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Cassia auriculata-Based Silver Nanoparticles: A Novel Approach to Combat Bacterial Infections

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ABSTRACT

Background: Bioactive compounds extracted from *Cassia auriculata*, a plant with therapeutic uses, promote the reduction of Ag+ to AgNPs. By avoiding the use of potentially hazardous substances typically employed in chemical synthesis, the green synthesis methodology offers a more economically viable and sustainable way of manufacturing nanoparticles.

Methods: AgNPs were generated through the addition of an extract from *C. auriculata* leaves with a solution of silver nitrates. The dimension and form of the nanoparticles have been investigated using X-ray diffraction (XRD), ZETA Potential, Fourier Transform Infra-Red (FTIR), UV-VIS spectroscopy, and scanning electron microscopy (SEM) to validate the synthesis. By the agar well diffusion method, the antibacterial property of the generated AgNPs was measured against *B. cereus*, *P. aeruginosa*, *S. aureus*, and *E. coli*.

Results: AgNPs of *C. auriculata* were confirmed by UV-VIS spectroscopy analysis with a peak around 421nm. The antibacterial tests showed significant inhibition zones for all tested bacterial strains, with *S.aureus* exhibiting the highest sensitivity. Considerable restricting zones appeared for *E. coli*, *P. aeruginosa*, and *B. cereus*, demonstrating the AgNPs' broad-spectrum antibacterial effectiveness.

Conclusions: Effective antibacterial activity over Gram-positive, as well as Gram-negative bacteria, has been shown by the generated AgNPs from *C. auriculata* leaf extract. The variances in cell wall construction among different kinds of bacteria may be the root cause of the diversity in the inhibitory zones. The study underscores the potential of using plant-based synthesis to produce AgNPs with strong antibacterial effects.

Key-words: AgNPs, Antibacterial activity, *Cassia auriculata*, DLS-ZETA potential, FTIR, SEM, XRD

INTRODUCTION

On a global level, traditional medicines are the only solution to many health problems. In several parts of the world, conventional medical techniques have been used for generations to treat an array of human ailments.

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In India, conventional healthcare still is heavily dependent on the use of herbal remedies as medications, and over eighty percent of the global population depends on them [1]. Plants that use traditional medicines provide various therapeutic agents [2]. Since prehistoric times, the usefulness of medicinal plants in both the relief and avoidance of all sorts of maladies has been acknowledged [3]. Several phytochemical substances isolated from medicinal plants tend to dampen the growth of bacteria [4].

A revolutionary innovation that encompasses numerous academic fields, spanning biology, chemistry, materials research, physics, and medicine, is nanotechnology. The

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acronym "nano" is Greek for "dwarf," and nanoparticles are 10^{-9} m in size. Materials with nano dimensions (1–100 nm) are significantly more reactive than conventional substances with the same compositions [5]. Targeted therapies using nanoparticles reduce the need for high doses, which results in fewer expected side effects of a drug [6]. AgNPs, which are used in various kinds of industries such as photovoltaics, biological, and chemical sensors, have specific optical, electrical, and thermal characteristics [7]. Researchers have developed a variety of synthetic nanoparticle production methods that have revealed a significant advantage to nature and the environment through safe, non-toxic, and environmentally sound "green chemistry" techniques that include creature's plants, a fungal infection, and microorganisms [8]. Some of the noble metal nanoparticles that have gained the most fascination are AgNPs partly because of their multitude of applications in food manufacturing, healthcare, dentistry, pharmaceutical delivery, tissue and tumor imaging, biolabeling, biosensing, optics, coatings for solar energy absorption, mirrors, photography, electronics, and pharmaceuticals [9]. A lot of potential has been expressed for employing plants for the manufacture of AgNPs. As opposed to the costly process of maintaining cell culture needed to perform bacterial synthesis of AgNPs, plant-mediated synthesis of AgNPs offers a variety of advantages, may be executed at room temperature at an economical cost, and is quite quick [10]. Because plants hold an array of metabolites that can reduce silver ions and stabilize and cap AgNPs, the quantity and form of AgNPs will vary depending on the species of plant. This is particularly true because medicinal plants are bountiful in a variety of phytonutrients and antioxidants [11]. Complex biomolecules contained in medicinal plants aid in the stabilization of nanoparticles into appropriate shapes and sizes and a decrease in metal ions. All that is required for generating AgNPs from plants is a plant extract and silver salt, which is thereafter reduced [12].

In this study, we selected *Cassia auriculata (C. auriculata)* leaves as a phytochemical source because the potential of this plant compound has been reported previously. Tanners Senna is another name for the Avaram tree. Similar regional names include Avartaki, Pitapuspa, Pitkalika, Manojyna, Pitkala, Charmaranga (Sanskrit), Tarwar, Awal, Tarval (Hindi), Tangedu, Merakatangeedu (Telugu), Arsual, Taravada, and Tarwad (Marathi) [13]. According to previous reports, the plant exhibits antipyretic, hepatoprotective, antidiabetic, antiperoxidative, antihyperglycemic, and microbicidal activities. The antibacterial and antifungal properties of *C. auriculata* are revealed by its methanol, chloroform, and aqueous extracts [14]. It has been demonstrated that *C. auriculata* contains antiviral and antispasmodic properties [15]. A UV-VIS spectrophotometer, FTIR, XRD, DLS, and ZETA potential analysis were implemented to characterize the produced AgNPs. SEM was employed for collecting morphological images of the nanoparticles.

MATERIALS AND METHODS

Recognition and collection of plant- Raw chemicals of analytical grade (AR) of silver nitrate ($AgNO_3$) of Rankem (CAS No. 7761-88-8) were collected from the Department of Zoology, University of Rajasthan, Jaipur. The fresh and healthy leaves of *C. auriculata* were collected in and around Jaipur city. The specimen voucher was verified at the Department of Botany, University of Rajasthan, Jaipur with a herbarium number RUBL21232**.** To get the plant extract, its leaves need to be washed, shade-dried, and sterilized according to specified guidelines.

Obtaining an extract using the leaves of plants- Following a shade-drying process, 50g of powdery *C. auriculata* leaves was mixed with 350ml of methanol and 350ml of double-distilled water (50:50), and the combination was Soxhlet for 8x3hours at 45–50°C [16]. After cooling and straining the sample through Whatman No. 1 filter paper, the plant extract was then collected in an airtight container and placed to dry in the hot oven at room temperature.

Synthesis of AgNPs by using silver nitrate and plant extract- Plant extract and 1 mM $AgNO_3$ solution were placed in a conical flask, which was followed by heating to 60–70ºC for 30–45 minutes. The synthesis of AgNPs was apparent by the reaction mixture's hue transforming from opaque to brown. It illustrates how the silver is reduced into AgNPs by the UV-VIS spectrophotometer [17].

Scanning of NPs and Antibacterial activity- The *in-vitro* antibacterial assay was carried out using the standard microbiological procedure, the Agar Well Diffusion

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method [18]. Three distinct concentrations of all chemicals the various samples were mixed with 10% dimethyl sulphoxide (DMSO). Disinfected Petri dishes holding the nutrient agar (NA) medium were used for the inoculation of test microorganisms, this inoculum spread all over the dish using a spreader and kept to stand for 30 min. In the implanted agar plates, wells of 6 mm in diameter have been formed. The regular medication was also added to a different petri dish in comparable quantities. All different concentrations of all the samples and standard drug (30µl) were poured into the pre-organized wells of seeded plates. For 24 hours, the plates were left to incubate at 37ºC. Each prepared well's inhibition zone (IZ) was used for examining the test sample's (100 mg/L, 200 mg/L, and 300 mg/L) were obtained after antibacterial spectrum. Inhibition zone dimensions generated through the test sample and generic antibiotics (streptomycin) were assessed.

Statistical analysis- Each experiment is repeated twice. Mean and standard deviation (SD) are used to represent the results in this study. To figure out if there were any statistically significant differences among the tested microorganisms, a one-way ANOVA test was employed. GraphPad PRISM 8.3 software (GraphPad, La Jolla, CA, USA) was applied for data analysis. A p-value of less than 0.05 was deemed statistically significant. The data is presented as mean ± SD.

RESULTS

UV-VIS spectrophotometer- For analysis of the UV-VIS spectrum, samples were prepared and experiments were conducted for each plant extract solution treated with $AgNO_3$ by varying concentrations of $AgNO_3$ and plant extract. A thermoscientific multiscan Go spectrophotometer at the Department of Zoology, University of Rajasthan, Jaipur, is employed for UV-VIS spectroscopy, which proves the synthesis of AgNPs in the 300–700 nm range. It reveals the presence of silver ion traces that are observable due to bio-reduction from silver oxide. Indicative of the formation of plant-mediated AgNPs, Fig. 1A depicts an apparent shift in color from yellowish to brown in the plant extract. The UV-Vis spectral analysis of *C. auriculata* AgNPs revealed an absorption maximum of 421 nm (Fig. 1 A-C).

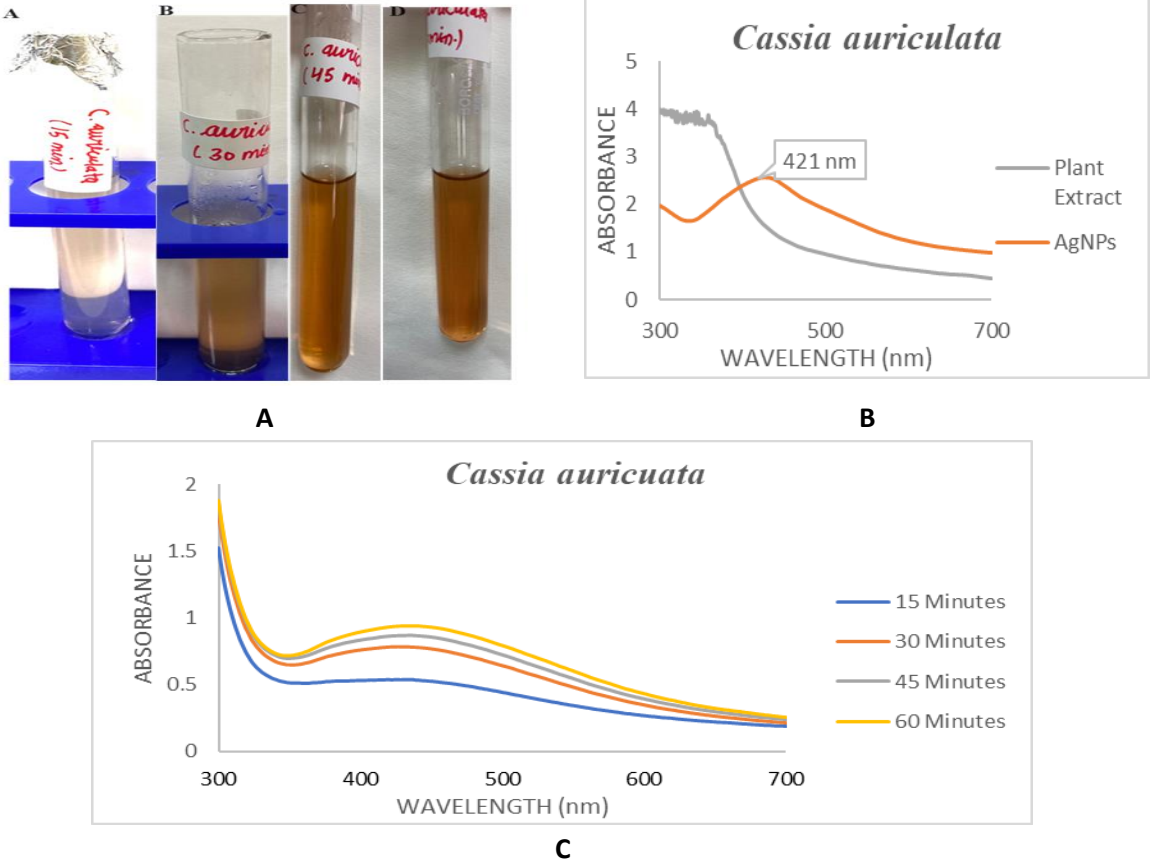

Fig. 1 (A-C): UV results of *Cassia auriculata* (A) Change in color with time change (B) Comparison with plant extract & AgNPs (C) Change in absorbance with change in time

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FTIR (Fourier- Transform Infrared)- The infrared (IR) spectrum of *C. auriculata* leaves in methanol as a solvent and the centrifuged AgNP sample were used to figure out the possible chemical constituents involved in the synthesis and capping of AgNPs. The samples were examined with the PerkinElmer Spectrum Version 10.4.00 at MRC MNIT Jaipur. The background was a KBr pellet that was 100 percent pure. The spectra were captured between 450 and 4000 cm^{-1}. The FTIR spectra of dried *C. auriculata* AgNPs have been linked with the key compounds to explore the phytochemical elements involved in the reduction and capping of AgNPs.

The nature of vibration, magnitude, and functional group composition of AgNPs is attributed to the wave number or frequency (cm-1) of the absorption band or peak when they are manufactured using *C. auriculata* leaf extract (Fig. 2A). Different functional groups were required to facilitate the transformation of silver ions to AgNPs.

The peaks in the range of 3400 to 3200 cm^{-1} and 3000 to 2850 cm^{-1} were ascribed to the aldehydic -C-H stretching of alkanes and the O-H stretching of alcohol and phenol compounds. IR peaks appeared at 3434cm^{-1}, 2924cm^{-1}, 2853cm^{-1}, 2071cm^{-1}, 1654cm^{-1}, 1637cm^{-1}, 1384.20cm^{-1}, 669.38cm^{-1}. Primary and secondary amine groups are shown by the strong broadband that emerged at 3434 cm^{-1}, which is ascribed to the N-H bend. Halo compounds C-I are represented by the medium bands at 2960 cm^{-1} alkane C-H, 1654 cm^{-1} conjugated alkene C=C, 1384 cm^{-1} carboxylic acid O-H, and 669 cm^{-1}. Fig. 2B demonstrates the FTIR spectra of a plant extract of *C. auriculata*. The peak at 2950 cm^{-1} implies the C-H stretching mode, the amide group at 1409 cm^{-1}, the C=O stretching mode at 1645 cm^{-1}, the existence of the COO-group at 1409 cm^{-1}, and the C-N aliphatic group at 1113 cm^{-1}, respectively.

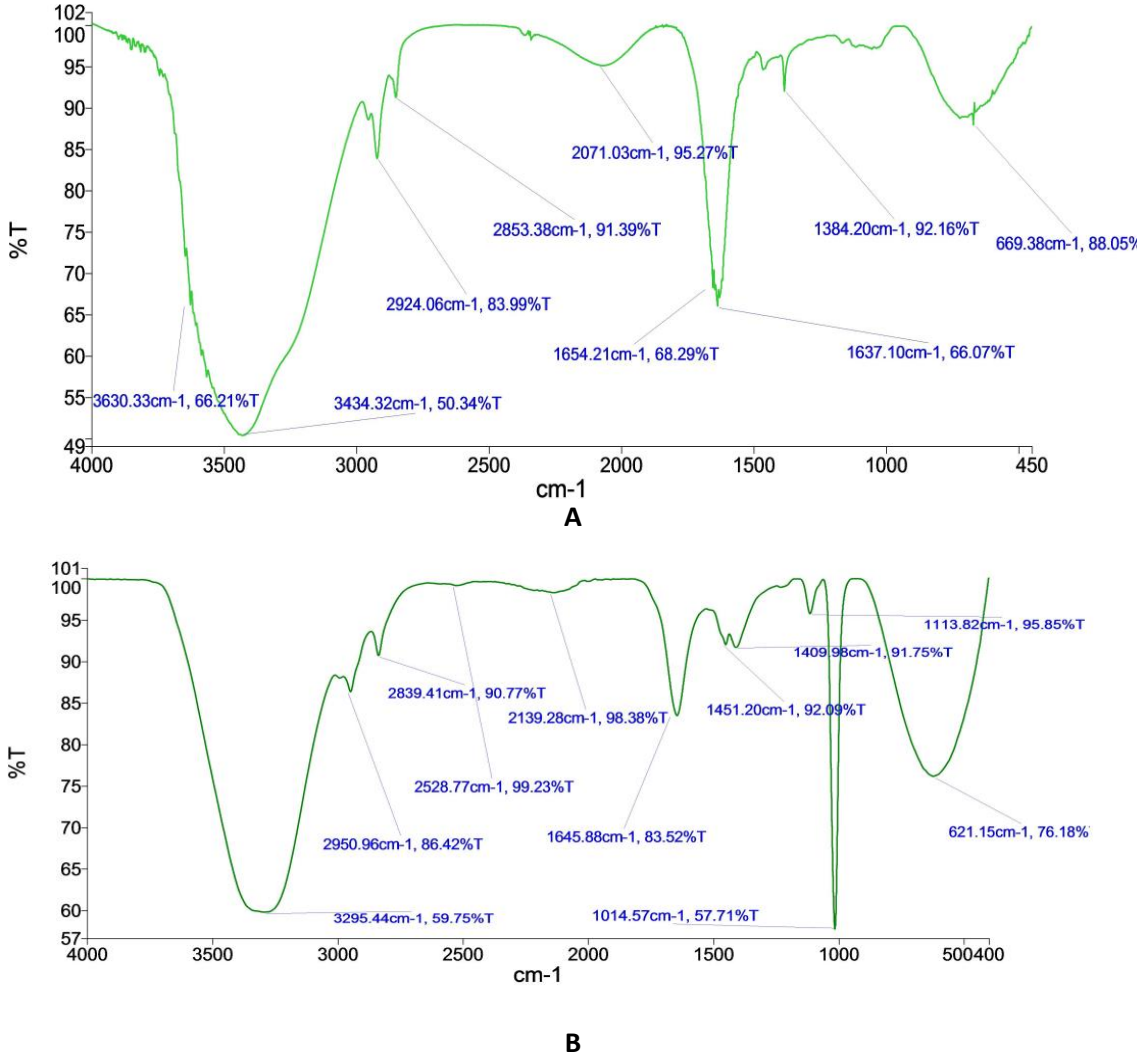

Fig. 2 (A,B): FTIR spectra of (A) AgNPs of *Cassia auriculata,* (B) Plant extract of *Cassia auriculata*

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XRD (X-Ray Diffraction)- The nanoparticles' crystal structure and diffraction pattern identification are done using XRD. The crystallographic planes that are peculiar to AgNPs are (100), (142), (231), (241), and (311). X-ray crystallography verified the produced AgNP crystal structure. With the aid of an XRD device, the nanoparticles' XRD pattern was examined and is depicted in Fig. 3.

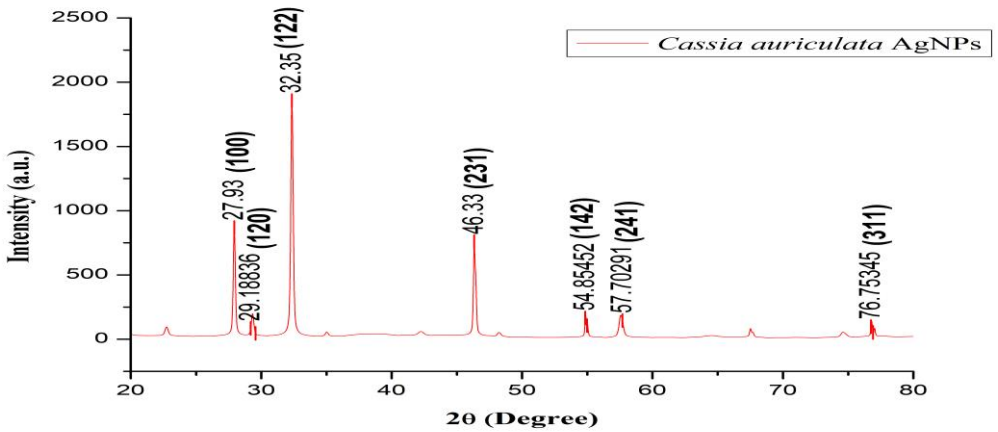

Fig. 3: XRD results of *Cassia auriculata*

Table 1: The crystallite size of AgNPs of *Cassia auriculata*

SAMPLE	2θ(°)	Hkl	FWHM (°)	D (nm)
AgNPs	27.93	100	0.1574	51.988
	29.18	120	0.1968	41.696
	32.35	122	0.1771	46.688
	46.33	231	0.1378	62.682
	54.85	142	0.1181	75.755
	57.70	241	0.2755	32.910
	76.75	311	0.1181	85.770
Mean				56.784

Applying Debye Scherrer's equation, the mean size of the crystallites has been calculated to be 56.784 nm (Table 1).

$$D \text{ (Crystalline size)} = K\lambda / \beta \cos\theta$$

here λ corresponds to the X-ray source's wavelength (0.154 nm), β denotes FWHM, θ is Bragg's angle, and K equals Scherrer's constant (0.9).

ZETA Potential- A conductivity of 0.157 mS/cm and a mean voltage of -25.9±3.98 mV was detected using the ZETA potential assessment to measure surface charge (Fig. 4). Functional groups from the *C. auriculata* extract imparted a relatively high negative charge value onto the nanoparticle's surface. This charge would have induced a negative–negative repulsion that would result in the AgNPs' high dispersity, colloidal nature, and durability over time. This activity was performed by using Malvern Instrument Ltd. Zetasizer Ver. 7.11 at MNIT MRC Jaipur.

DLS (Dynamic Light Scattering)- *C. auriculata*-derived AgNPs had a PDI of 0.439 and an average diameter of 139.9nm, (Fig. 5) indicating that they were polydispersed, according to the particle size distribution curve. This activity was performed by using Malvern Instrument Ltd. Zetasizer Ver. 7.11 at MNIT MRC Jaipur.

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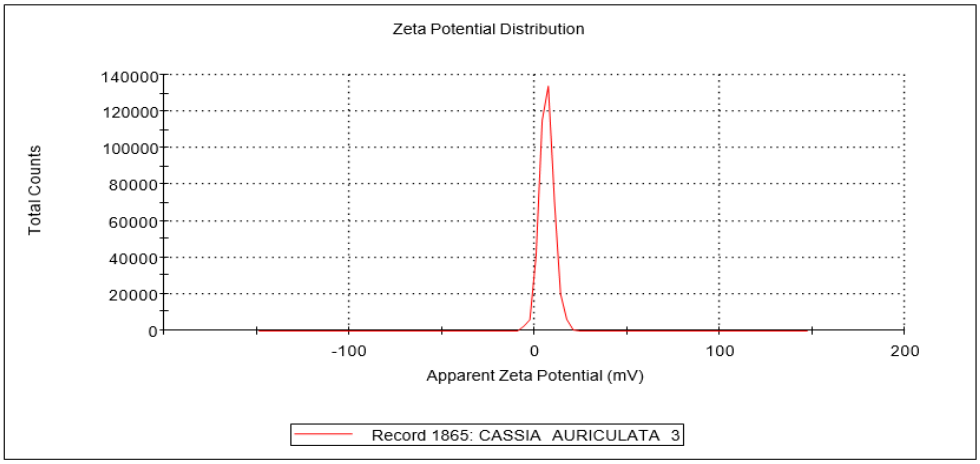

Fig. 4: ZETA Potential of *Cassia auriculata*

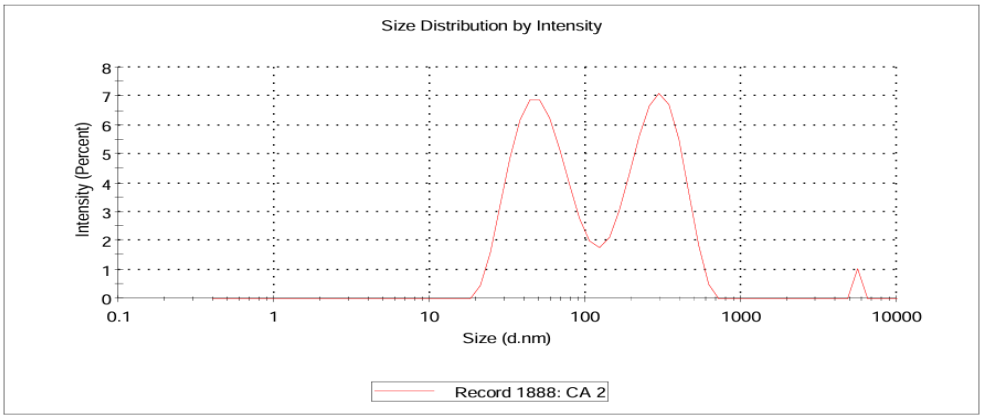

Fig. 5: DLS of *Cassia auriculata*

SEM (Scanning Electron Microscope) and EDX (Energy Dispersive X-ray spectroscopy)- To locate every aspect of the biogenic AgNPs, energy-dispersive X-ray spectroscopy (EDX) research is conducted. AgNPs generated from methanolic *C. auriculata* leaf extract revealed the following elements within their EDX spectra and SEM graph: silver (41.83%), Cl (7.54%), P (3.49%), Mg (3.35%), Zn (2.45%), Mn (0.42%), and Fe (0.37%). SEM photographs of AgNPs synthesized with leaf extract from *C. auriculata* indicate that most of the particles are spherical, smooth, and distributed uniformly in a compact configuration. The range of the mean size of the particles has been estimated to be 10–40 nm. SEM was done by using NOVA NANOSEM450 at HV 15.0kv and EDX was done by using a BRUKER energy dispersive X-ray analyzer at MNIT MRC, Jaipur.

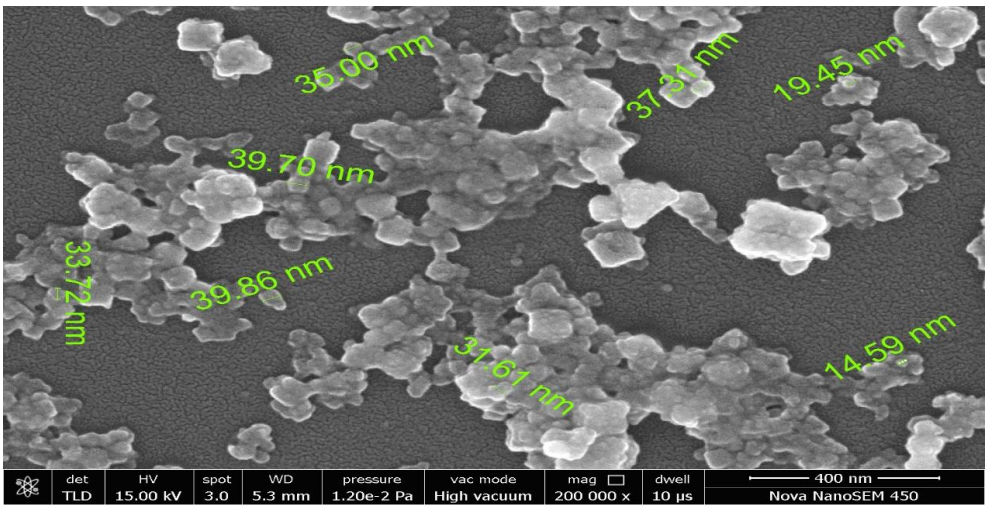

Fig. 6: SEM image of *Cassia auriculata*

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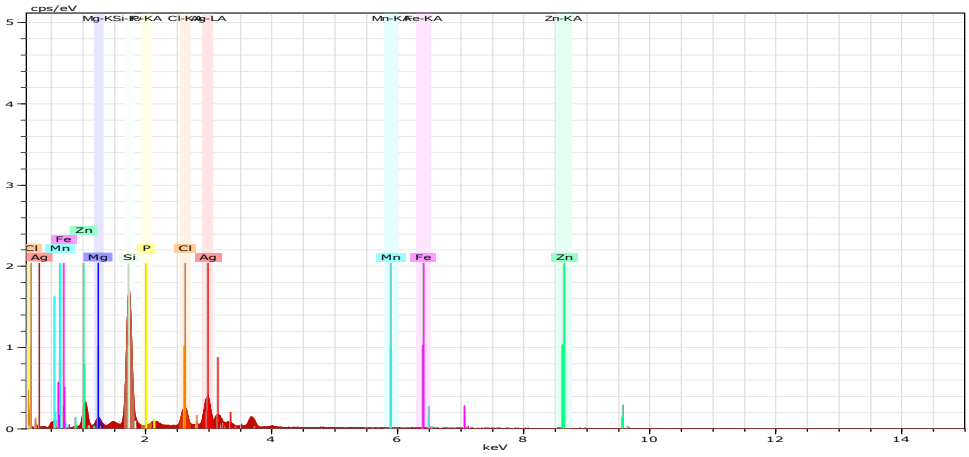

Fig. 7: EDX analysis of *Cassia auriculata*

AgNPs antibacterial effectiveness against pathogenic bacterial strains of gram-positive *S. aureus* and *B. cereus* along with gram-negative *E. coli* and *P.aeruginosa* has been studied utilizing the well diffusion method. The control group was composed of common antibiotics like Streptomycin, plant extracts, $AgNO_3$, and distilled water. From research on antibacterial activity, all the generated AgNPs displayed strong antibacterial properties against gram-positive *S. aureus* as well as gram-negative *E. coli* bacterial strains. The zone of peak performance inhibition was detected in *S.aureus* measuring 15±1.414mm (100mg/L), 18.5±0.707mm (200mg/L), and 23±1.414mm (300mg/L) followed by gram-negative bacteria *E. coli* strain measuring 12±1.414mm (100mg/L), 14.5±0.707mm (200mg/L) and 16.5±0.707mm (300mg/L) and minimum zone of inhibition was observed in *P. aeruginosa* measuring 3±0.707mm (100mg/L), 5.5±0.707mm (200mg/L) and 8±1.414mm (300mg/L).

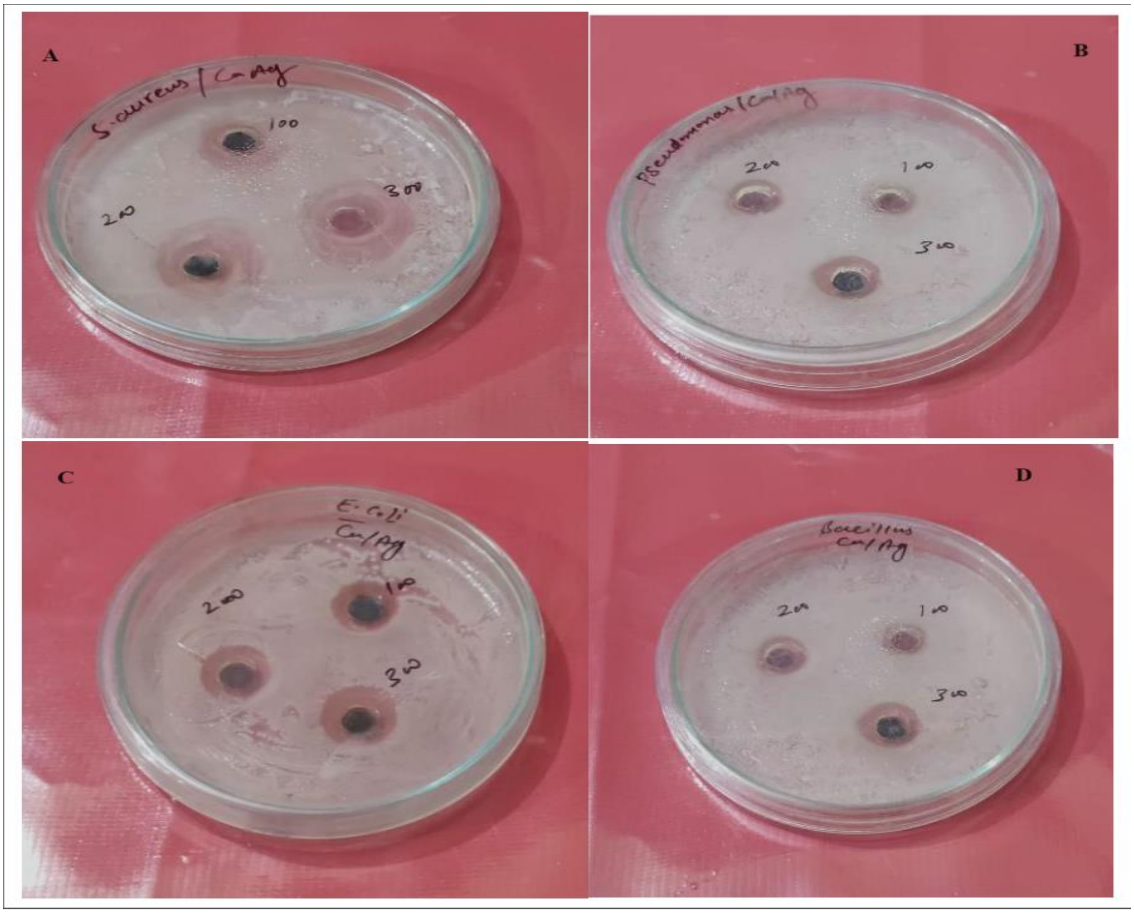

Fig. 8: Antibacterial activity of AgNPs of *Cassia auriculata* (A) *S. aureus* (B) *P. aeruginosa* (C) *E. coli* (D) *B. cereus*

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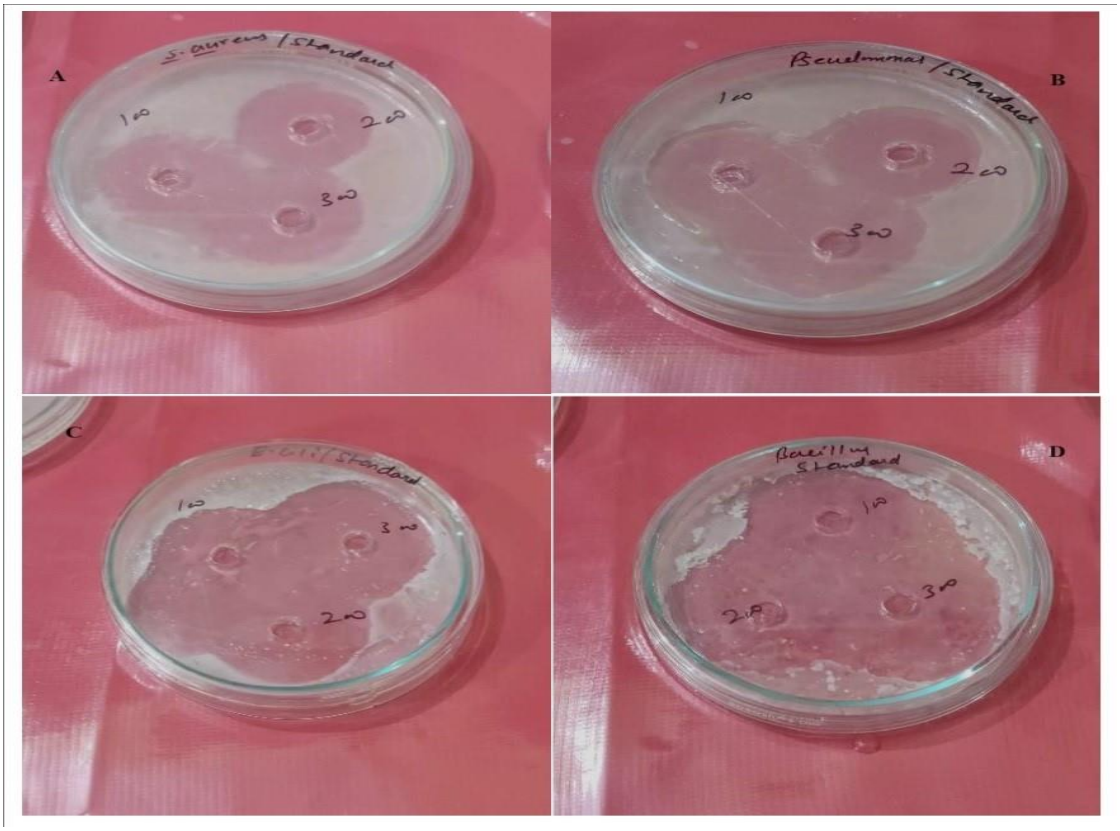

Fig. 9: Antibacterial activity of standard (Streptomycin) against (A) *S. aureus* (B) *P. aeruginosa* (C) *E. coli* (D) *B. cereus*

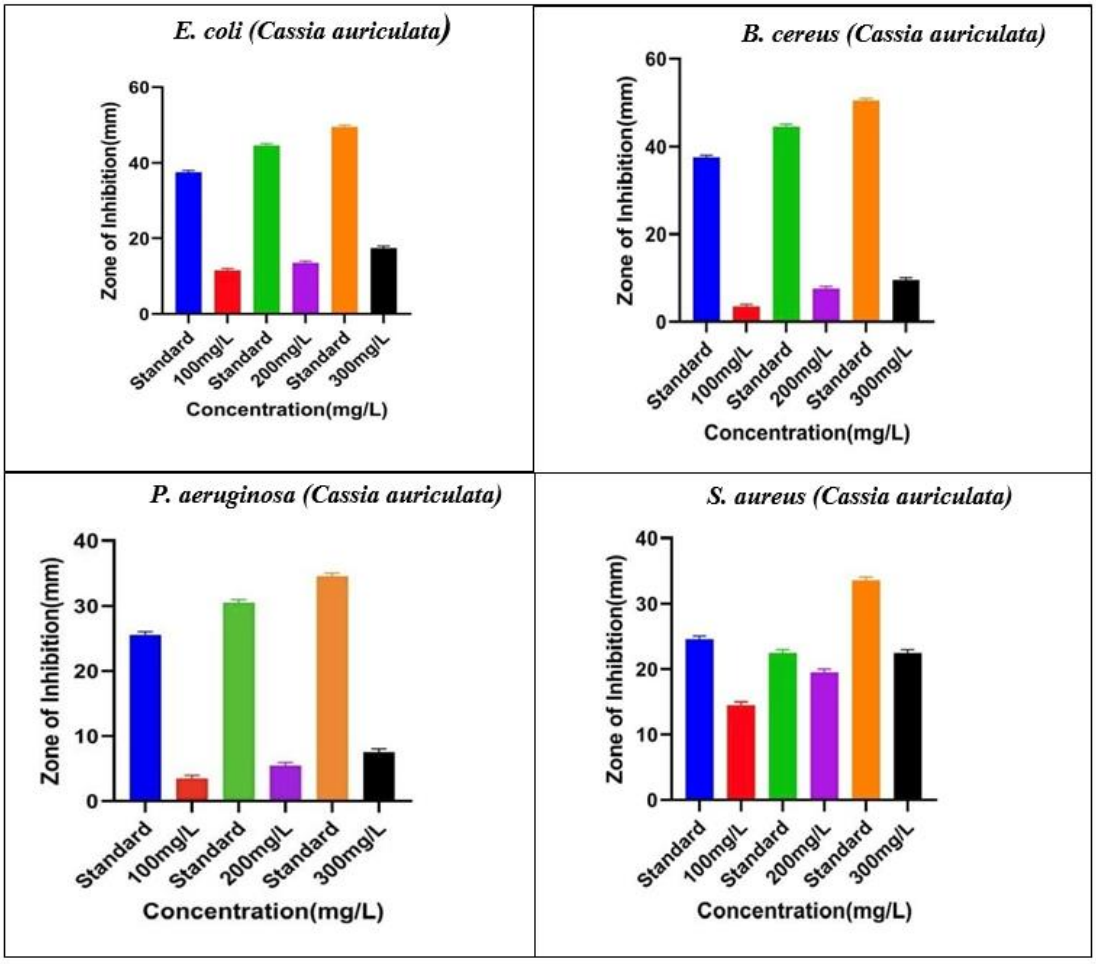

Fig.10: Statistical analysis of Bacterial strains which are treated with AgNPs of *Cassia auriculata*

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DISCUSSION

In this research, researchers utilized a methanolic extract of *C. auriculata* to successfully generate AgNPs. Different approaches to analysis, which include, UV-VIS spectroscopy, FTIR (Fourier-Transform Infra-Red), XRD, DLS-ZETA potential, and SEM were implemented to validate the characterization of the generated AgNPs. There was a considerable absorption peak in the UV-Vis spectrum at 421 nm, which suggested that AgNPs had been synthesized. A visual shift is also apparent in UV-VIS spectroscopy after 30 minutes of the reaction process, as the solution mixture's hue changes from a translucent blue to dark brown. One possible explanation for the noted shifts in solution color is the occurrence of bilateral electromagnetic fields by the coordinated oscillations of free electron populations in the surface plasmon resonance phenomenon [19]. A prominent spectroscopic tool for confirming the generation of AgNPs in a colloidal solution through the surface plasmon resonance phenomena of metallic nanoparticles is the UV-VIS spectrophotometer. The synthesized nanoparticles' size, shape, concentration, and aggregation state all impact this optical feature [20]. Nanoscale silver particles tend to be sustained by phytochemicals through molecular contact with metal surfaces. According to the literature, FTIR analysis can be used to explore the kind of molecular interaction and its functionalities using the functional group reference peaks [21]. The FTIR spectrum suggested a range of phytochemical components in the leaf extract, namely anthraquinones, flavonoids, terpenoids, phenols, tannins, polysaccharides, alkaloids, saponins, and cardiac glycosides [22]. The XRD research indicated the nanoparticles appeared crystalline, with visible peaks that suited the face-centered cubic structure of silver. Characterization of AgNPs deploying Bragg's reflection using XRD is significant [23]. The colloidal stability of nanoparticles is influenced by their surface charges, which are revealed by zeta potential studies [24]. The bell-shaped pattern of the size distribution by intensity reflects the extensive range of nanoparticle forms in the sample material. Nanoparticle variation in size and charging at the surface while drifting in a liquid are quantified using DLS [25]. Uniformly scattered spherical nanoparticles with a size range of 15–35 nm was evident in SEM pictures. A scanning electron microscope and energy-dispersive X-ray spectroscopy were both employed to perform the elemental and morphological studies. For determining the elemental composition of the AgNPs, EDX spectroscopy was chosen. It may be reasonable to combine SEM and EDX to study the components and estimate the physical appearance of AgNPs. According to EDX, which also demonstrated that pure silver was abundant in the chemical makeup of metal nanoparticles, the strong optical absorption peak at 15.0kv was caused by surface plasmon resonance, which was produced by silver in the nanocrystalline nature. One major drawback of SEM is its inability to identify interior structures, even though it can offer valuable insights into the type and extent of nanoparticle aggregation [26].

AgNPs' antibacterial property was tested against four bacterial strains: *P. aeruginosa*, *B. cereus*, *S. aureus*, and *E. coli.* The results demonstrated significant antibacterial activity, which varied among the different bacterial strains. The zone of inhibition assays indicated that *S. aureus* and *E. coli* were inclined to the AgNPs compared to *B. cereus* and *P. aeruginosa*. The gram-positive and gram-negative bacteria's varied cell wall architectures may be the reason for this discrepancy in susceptibility; gram-positive bacteria's thicker peptidoglycan layer might make way for enhanced communication with AgNPs.

The mechanism underlying the antibacterial activity of AgNPs involves multiple pathways. AgNPs can primarily link to the cell membrane of the bacteria wall, disrupting its structure and enhancing its permeability, triggering cell lysis. Additionally, AgNPs can penetrate bacterial cells that are in contact with intracellular regions, generating reactive oxygen species (ROS) and disrupting cellular functions such as DNA replication and protein synthesis. The observed higher efficacy against gram-positive bacteria suggests that a vital variable in AgNPs' effectiveness against bacteria is how they connect with various parts of the cell wall.

Moreover, the biosynthesis of AgNPs using *C. auriculata* extract is advantageous considering it's affordable and good for the environment and the presence of phytochemicals that can boost the nanoparticles' resilience and antimicrobial qualities. The green synthesis approach aligns with sustainable practices and offers an optimistic choice to conventional physical as well as chemical approaches to generate nanoparticles. The AgNPs displayed dose-dependent antibacterial

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activity, with higher concentrations of nanoparticles leading to larger zones of inhibition. The minimum inhibitory concentration (MIC) values were consistent with the agar diffusion results, adding to the generated AgNPs' remarkable antibacterial capabilities.

CONCLUSIONS

The research investigation culminated by accurately showing the generation of AgNPs employing leaf extract from *C. auriculata* showcasing an economical and sustainable technique. Strong antibacterial activity has been shown by the generated AgNPs against both Gram-positive and Gram-negative bacteria, with *S. aureus* being particularly responsive. The findings suggest that these biosynthesized AgNPs might be put to use in an array of ways as strong antibacterial drugs, including medical devices, wound dressings, and water purification systems.

Future research should focus on elucidating the detailed mechanisms of antibacterial action, measuring these nanoparticles' sensitivity in human cells, and exploring their efficacy *in vivo* models. Additionally, their antibacterial characteristics could be enhanced more by manipulating the synthesis parameters to regulate the size and shape of the nanoparticles.

ACKNOWLEDGMENTS

We thus affirm that owing to the IUCN, the plant under consideration (*C. auriculata*, an evergreen shrub) is not a species at risk of extinction. Research on this plant accords with national, international, and institutional norms. The Head of the Zoology Division at the University of Rajasthan in Jaipur, India, and the Council of Scientific and Industrial Research (CSIR) in New Delhi have both granted financial support, which the authors acknowledged.

CONTRIBUTION OF AUTHORS

Research concept- Prity Yadav
Research design- Prity Yadav
Supervision- Dr. Pratap Chand Mali
Materials- Neha Bharti
Data collection- Prity Yadav, Neha Bharti
Data analysis and interpretation- Prity Yadav, Neha Bharti
Literature search- Prity Yadav, Neha Bharti, Ashish Kumar Kansotiya
Writing article- Prity Yadav
Critical review- Dr. Pratap Chand Mali
Article editing- Prity Yadav
Final approval- Dr. Pratap Chand Mali

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