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GREEN SYNTHESIS OF SILVER NANO PARTICLES BY USING CELASTRUS PANICULATUS LEAF, SEED EXTRACTS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

Nanoparticle research is a promising field due to its diverse biological applications. Green synthesis, a cost-effective and sustainable method, involves synthesizing nanoparticles from organisms like fungi, algae, yeast, bacteria, and microbial enzymes, resulting in significant outcomes. Our study's primary goal is to investigate the process of synthesizing silver nanoparticles using *Celastrus paniculatus* leaf and seed extracts using environmentally friendly methods. The produced silver nanoparticles (AgNPs) from leaf (AgNPs-L) and seed (AgNPs-S) extracts were analyzed using Ultraviolet-Visible spectroscopy, and Fourier-transform infrared (FTIR). X-ray diffraction analysis (XRD), scanning electron microscopy (SEM) and also evaluated their antibacterial efficacy against three distinct strains. The UV-visible spectroscopic examination of the synthesized AgNPs-L shows maximum absorption peak at 419 nm and AgNPs-S at 425 nm at 423 nm, confirming the existence of surface plasmon resonance. The X-ray diffraction examination unveiled the crystal structure of the produced AgNPs, SEM confirms the particles sizes ranges 40nm to 150nm. *S.aureus* (MTCC-96), *E.coli (*MTCC-443) showed zone of inhibition (ZOI) of 21±0.60 mm and 12.6±0.81, with 25 and 10 µg/ml concentration of AgNPs-L which is maximum as compare AgNPs-S (19.13±0.30) and (10.63±0.37). Hence, the AgNPs generated via the process utilizing leaf extract exhibited strong antibacterial activities than AgNPs-S generated by seed extracts.

KEYWORDS: Antibacterial; *Celastrus paniculatus*; Green synthesis; SEM; Silver nanoparticles.

INTRODUCTION

Nanotechnology involves synthesizing particles with a high surface to volume ratio of 1-100 nm size, resulting in unique physical, chemical, and biological properties.[1] These properties enhance the interaction with biological systems effectively, which makes them ideal for use as antioxidants, anticancer agents, and antibiotics.[2,3] Recent studies on noble metal nanoparticles show that they include gold, silver, platinum, and palladium nanoparticles, which have extensive applications in cancer treatment.[4] Gold and silver nanoparticles are extensively researched for their potential in diagnostics, drug delivery, and therapeutic agents due to their biocompatibility and ease of fictionalization.[5] Silver nanoparticles (AgNPs) are widely used due to their unique properties and potential applications in pharmaceuticals, agriculture, water detoxification, air filtration, textile industries, and oxidation reactions.[6,7] There are three main methods for synthesizing the silver nanoparticles such as physical, chemical and biological synthesis, and each has its own set of advantages and limitations.[8] The challenges of size control, particle size uniformity, and cost in physical synthesis have led to a shift towards controlled chemical synthesis.[9] However, chemical synthesis involves harsh reaction conditions, environmental pollution due to chemical waste, and the presence of impurities in the final product.[10]

The field of green chemistry has advanced by employing plant extracts that contain a variety of metabolites to convert silver ions into silver nanoparticles and then encapsulate them to improve their longevity.[11] The primary emphasis has been on the production of silver nanoparticles by the utilization of plant leaf ,[12] seed,[13] flower,[14] stem,[15] callus,[16] hairy roots extracts.[17] The current study focused on the *Celastrus paniculatus* plant, which is popularly referred to as the 'Tree of Life' in Ayurvedic medicine. This plant is a member of the Celastraceae family and is commonly referred to as Jyotishmati, Kangani, and Malkangni. It originates from India and is categorized as a Medhya Rasayana. It has been proven to possess neuroprotective qualities. Additionally, it displays antioxidant, anti-inflammatory, antibacterial, antidepressant, antidiabetic, hypolipidemic, antirheumatic, memory-enhancing, analgesic, antiepileptic, anticonvulsant, sedative, and anticancer

effects.[18,19] The phytochemical composition of this plant consists of polyalcohols, phenylpropanoids, saponins, alkaloids, tannins, glycosides, coumarins, flavonoids.[20,21] The earlier study involved the production of AgNPs using the aerial extract of *C. paniculatus* by a green synthesis method. The study investigates the antioxidant, anti proliferative, and anti hemolytic characteristics of silver nanoparticles produced utilizing extracts from Simarouba glauca and *C. paniculatus* plants.[22] Another study presents a method for producing AgNPs using leaf and callus extracts. Both extracts demonstrate strong antibacterial properties, suggesting that AgNPs derived from this medicinal plant could be valuable for developing new drugs.[23] In the present study, we sought to develop a fast, novel, and eco-friendly approach for the biogenic synthesis of AgNPs by employing *C. paniculatus* leaf and seed extracts as a reducing agent.

MATERIALS AND METHODS

Preparation of Leaf and Seed extracts and biosynthesis of nano materials

C. paniculatus Plant was collected at Matloddi village, kuntala mandal, adlabad district, Telangana in 2022. The plant leaves and seeds were isolated, rinsed with tap water, cut into small fragments, and pulverized using mechanical grinding. The process for preparing 10% leaf and seed extracts is as follows: A quantity of 10 grams of powder was mixed with 80 milliliters of double distilled water. The final volume was adjusted to 100 ml. The mixture was then incubated at a temperature of 65 °C for duration of 1 hr. After incubation, the extracts were filtered individually using whatman No-1 filter paper. The filtered extracts were then stored in separate collecting flasks at a temperature of 4 °C Utilized as a reducing and stabilizing agent in the synthesis of AgNPs. The process involved mixing a 2 mL, 10% plant extract with a 100 mL, 1 mM aqueous silver nitrate solution. The mixture was then incubated at a temperature of 60 °C. The successful creation of silver nanoparticles was confirmed by the appearance of a brown color.

Characterizations of obtained AgNPs

Uv-visible spectrophotometery analysis

The formation of nanoparticles was promptly noticed with the addition of leaf extract, and we measured the absorbance at 0, 10, 20, and 30 min. The generation of AgNPs was evaluated by collecting samples at 0, 30, 60, and 120-min intervals where seed extract was utilized as a reducing agent. The analysis was conducted using an Eppendorf Biospectrometer kinetic spectrophotometer. The surface plasmon spectra of both synthesis processes for AgNPs were measured with a resolution of 1 nm, ranging from 280 to 800 nm.

FTIR analysis of AgNPs

Analysis of AgNPs was conducted using Fourier transform infrared spectroscopy (FTIR-Agilent) using a sample consisting of finely air-dried powder. A 2 mg sample was combined with KBr powder and an infrared (IR) spectrum was recorded. The resolution range was set at 450–4000 cm^{-1} and the intensity was set at 4 cm^{-1}. The analysis revealed the presence of various functional groups in the leaf and seed extracts of *C. paniculatus*, which are believed to play a role in the synthesis reaction of AgNPs.

X Ray Diffraction evaluation (XRD)

The AgNPs solution obtained from leaf and seed extracts of *C. paniculatus* were subjected to centrifugation at a speed of 12000 rpm for duration of 10 minutes. The resulting pellet was then washed three times with distilled water, followed by drying at a temperature of 65 °C for a period of 2 hours. This process yielded a powdered form of AgNPs. The size and structure of silver nanoparticles in their dried powdered state were studied using the Shimadzu Maxima_X XRD-7000. A recorded X-ray diffraction pattern was used to confirm the creation of AgNPs, utilizing an X-ray Diffractometer.

Scanning Electron Microscopy and EDAX

The shape, size, and composition of AgNPs synthesized by both the methods were analyzed using SEM-EDAX. Sample preparation involved taking the synthesized AgNPs powder into an eppendorff tube, adding milliQ water, and sonicating it at room temperature for 30 minutes. Then, 40 µl of the particles were placed on a cover slide and air dried for 2 hours. The cover slides were positioned over a 200-mesh copper-grid that had been coated with carbon tape and then allowed to evaporate. The images were obtained using the Carl Zeiss Evo 18 Research 30 kV microscope equipped with a SE detector and EDAX.

Bacterial Strains and Antibacterial activity of synthesized nanoparticles

In the study, antimicrobial assay was performed by taking gram-positive *Staphylococcus aureus* (MTCC-96), gram-negative bacteria *Klebsiella aerogenes* (MTCC-111), and *E. coli* (MTCC-443) strains, which were obtained from the laboratory, stored stock cultures. The culture stocks were preserved by storing them in a solution consisting of 80% nutrient broth and 20% glycerol (v/v). The storage temperature was maintained at -80 °C. The glycerol stock cultures were streaked on Nutrient agar plates and cultivated overnight. Freshly obtained colonies were inoculated into nutrient broth incubated overnight. A volume of 1:100 dilutions made as follows, where 100 µl overnight grown cultures was inoculated in 1ml fresh nutrient broth medium incubated for 2 hrs. After two hours, the optical density (OD) was measured and subsequently corrected to a spread of 0.6 O D on freshly prepared nutrient agar plates. The agar well diffusion technique was employed to quantify the AgNPs produced by both the extracts the plant extract. Solutions containing AgNPs were introduced into wells that were made on Nutrient agar plates at concentrations of approximately 5, 10, 20, and 50µg/mL for the purpose of conducting antibacterial experiments. Subsequently, the plates were incubated for 24 hours at a temperature

of 37 °C. A 25µg/mL concentration of the antibiotic cefotoxime was utilized as a positive control. The diameter of the zone of inhibition was utilized to evaluate the size of the growth inhibition area.

RESULTS AND DISCUSSION

In both methods, AgNPs appear yellowish-brown in synthesized aqueous solutions as a result of surface plasmon vibrations. As the leaf extracts were added to the aqueous silver nitrate solution, the color of the solution changed from faint light to yellowish brown in 10 minutes, and finally the solution completely turned reddish brown in 30 minutes, indicating AgNPs formation. Similar changes in colour have also been observed in seed extract used silver nitrate solution but the final color change observed in 120 min indicating that the formation of AgNPs-S is slower than the leaf extracts AgNPs-L (Fig. 1A &1B). The final color confirms the completion of reaction between leaf and seed extracts with $AgNO_3$ solution. The UV-vis spectra recorded 0, 10, 20 and 30 min, for leaf extracts and 0, 30, 60 and 120 min for the seed extracts. The maximum Absorption spectra of leaf extract synthesized AgNPs were detected in between 410 to 430 nm and highest OD were detected at 419 nm (Fig. 1A), the peaks were in accordance with previous study where *Moringa oleifera* leaves extract demonstrated significant efficacy in rapidly reducing silver ions to synthesize AgNPs, The SPR peaks were observed at 415-439 nm, The shape, size, morphology, and composition of the synthesized AgNPs is directly influenced by surface plasmon resonance (SPR) bands.[24] The (SPR) shifted towards shorter wavelengths, resulting in the production of nanoparticles with reduced size. The smaller particles possess a greater surface area, hence enhancing their stability.[25] Whereas the maximum Absorption spectra of AgNPs synthesized by seed extract were detected in between 410 to 430 nm and maximum OD was recorded at 425 nm (Fig. 1B). The study reports the use of *Catharanthus roseus* seed extract for producing stable silver nanoparticles, with UV-Vis spectroscopy revealing the bio reduction of elemental silver at 425 nm, these results are inconformity with present study.[26,27]

The phyto components of the *C. paniculatus* extract that have the capacity to reduce and stabilize the produced AgNPs by capping were identified using FTIR spectrum analysis. This investigation provides more information on how AgNPs can be modified with other molecules for various purposes. The FTIR spectra of AgNPs-L' exhibited prominent absorption bands (Fig. 2A) at 3434, 3369, 3336, 3237, 3151, and 1418 cm^{-1}, corresponding to stretching vibrations of C-H, N-H, and O-H bonds. Additionally, absorption bands were observed at 1718, 1617, 1541, 1364, and 1032-762 cm^{-1} (Fig 2A). The following spectral peaks were observed: organic nitrates (1640–1620 cm^{-1}), aromatic C–H out-of-plane bend (900–670 cm^{-1}), secondary amine NH bend (1650–1550 cm^{-1}), alkyne C–H bend (680–610 cm^{-1}), and alkenyl C=C stretch (1680–1620 cm^{-1}). The C=C bond was identified as the medium sharp band at 1641 cm^{-1}, which suggests the stretching of the alkene group. The C=C bond had a prominent and strong peak at 896, 825, 773 cm^{-1}, indicating the bending movement of the alkane group that contains the C=C bond. These results are in collaborated with following studies where the synthesis of AgNPs using Aqueous Extract of *Typha domingensis* Pers. Pollen.[28,29] Whereas AgNPs-S exhibited peaks at 3392, 3259, 2920, 2849, 1638, 1399, 1321 cm^{-1} (O-H, N-H, C=C), (Fig. 2C), as compare with AgNPs-S the AgNPs-L as more hydroxyl group stretching's which is confirming the leaf extract has various bio active compounds presence of functional groups like carbohydrates, flavonoids, polyphenols, proteins, and terpenoids in leaf extracts of *C. paniculatus* compare with seed extracts, which act as reducing, capping, and stabilizing agents, facilitating the formation of silver nanoparticles with significant antibacterial properties.[23]

The X-ray diffraction technique was employed to study the crystalline structure and lattice properties of the produced AgNPs. The angle 2θ values vary from 20° to 100° utilizing Cu Kα radiation with a wavelength of 1.5406 A°. The AgNPs-L presence various peaks were verified through the examination of the XRD pattern, as depicted in Fig. 2B. The sample exhibits four clearly distinguishable major diffraction peaks at specific 2θ the angles 27.56°, 31.95°, 37.82 °and 45.99° correspond in the face centered cubic structure of silver. The findings of the study are consistent with previous results where AgNPs exhibited diffraction peaks at around 27° and 31°, respectively.[30,31] AgNPs-S also clearly displays four distinct peaks for 2θ values of 27.58°, 32.01°, 45.98°, 54.55° and 57.55° (Fig. 2D). The 2θ values correspond to the diffraction planes (220), (122), (231), (331), (241) and (311) the FCC structure of the produced AgNPs,[32] this represents the optimal orientation of the structure.[33] The produced AgNPs were analyzed using scanning electron microscopy (SEM), which revealed at magnification of 200 nm scale the presence of agglomeration, predominantly exhibiting a spherical in morphology some are cuboids (Fig. 3A & 3B). The both AgNPs-L and AgNPs-S size distribution is similar and ranging from 40 to 150 nm. EDAX analysis revealed the existence of elemental silver in the reaction mixture. The AgNPs-L and AgNPs-S displayed an optical absorption band with a peak at 3 keV, which is characteristic of the absorption behavior of metallic silver nanoparticles, confirms the presence of silver in the AgNPs-L and AgNPs-S (Fig. 4A & 4B).

During the extended analysis of the antibacterial activity of the biosynthesized AgNPs, distinct areas devoid of bacterial growth were detected surrounding the wells in plates that contained different quantities of AgNPs. The size of the ZOI was directly proportional to the concentration of AgNPs used in the study. *S. aureus* (MTCC-96), *E. coli (*MTCC-443) Plates with 25 and 10 µg/ml of AgNPs-L had a ZOI of 21±0.60 and 12.6±0.81 mm, which is a maximum activity exhibited by AgNPs-L

as compared to AgNPs-S (19.13±0.30) and (10.63±0.37), respectively (Fig. 5A & 5B). While *K. aerogenes (*MTCC-111) exhibited similar activity with both AgNPs-L and AgNPs-S at all four concentrations and the ZOI is represented in Table 1 &2. Compounds containing silver have been discovered to possess antibacterial activity against a range of bacteria, viruses, and fungi. Nevertheless, the effectiveness of AgNPs derived from plant extracts and preserved under normal environmental conditions has not been investigated.[34] The study found that bio synthesized AgNPs showed strong antibacterial properties due to protein capping, and also suggests that biologically produced AgNPs could be an eco-friendly alternative to ecologically sustainable substitute for AgNPs manufactured by chemical methods.[11] The study found that Ag NPs produced using silver nitrate and pomegranate peel extract showed potent antibacterial effects against *E. coli* strains. At varying concentrations (5, 10, and 15 µg), the inhibitory zone was disregarded, but at 10 and 15 µg, bacterial growth was significantly suppressed near AgNPs.[35]

Table 1: The results of antibacterial activity with zone of inhibition (mm) of synthesized AgNPs-L of *C. paniculatus* against microorganisms.

Bacterial species	Zone of inhibition (mean ± SD* in mm)				
	Synthesised AgNPs in concentration µg/ml				
	5	10	25	50	Ceftoxime
S.aureus (MTCC-96)	16.3±0.3	18.2±0.7	21±0.60	21.7±0.52	34.1±0.9
*E.coli (*MTCC-443)	11.16±0.7	12.6±0.81	18.03±0.650	20.26±1.0	30.26±0.2
*K. aerogenes (*MTCC-111)	9.4±0.1	11.6±1.07	12.86±0.25	14.83±0.3	28.63±0.15

Table 2: The results of antibacterial activity with zone of inhibition (mm) of synthesized AgNPs-S of *C. paniculatus* against microorganisms.

Bacterial species	Zone of inhibition (mean ± SD* in mm)				
	Synthesised AgNPs in concentration µg/ml				
	5	10	25	50	ceftoxime
S.aureus (MTCC-96)	11.96±0.47	17.13±0.35	19.13±0.30	20.2±0.36	33.36±0.32
*E.coli (*MTCC-443)	10.06±0.15	10.63±0.37	12.3±0.1	16.56±0.3	20.56±0.25
*K. aerogenes (*MTCC-111)	8.43±0.40	10.4±0.2	11.46±0.37	14.4±0.43	27.3±0.2

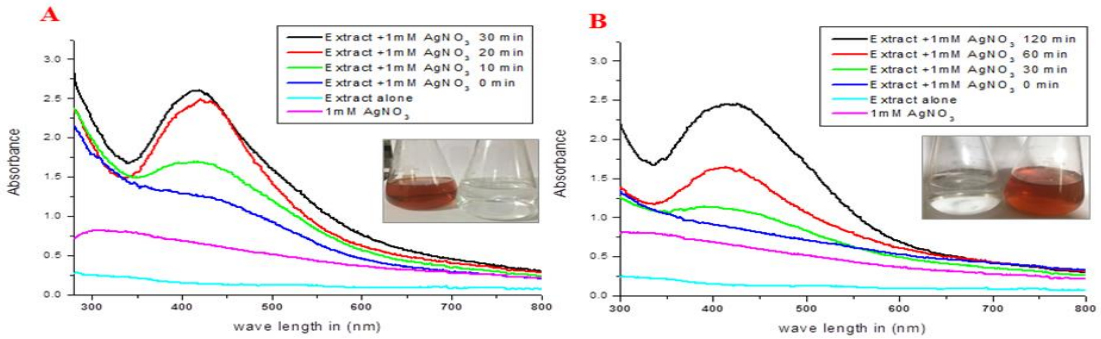
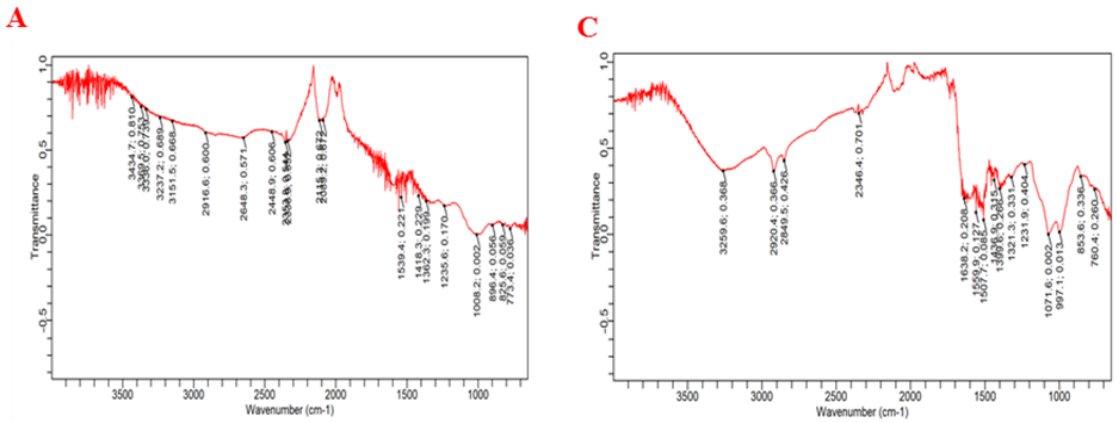


Fig. 1: AgNPs Characterization (A) Absorption spectra of solution with AgNPs-L; (B) Absorption spectra of solution with AgNPs-S.



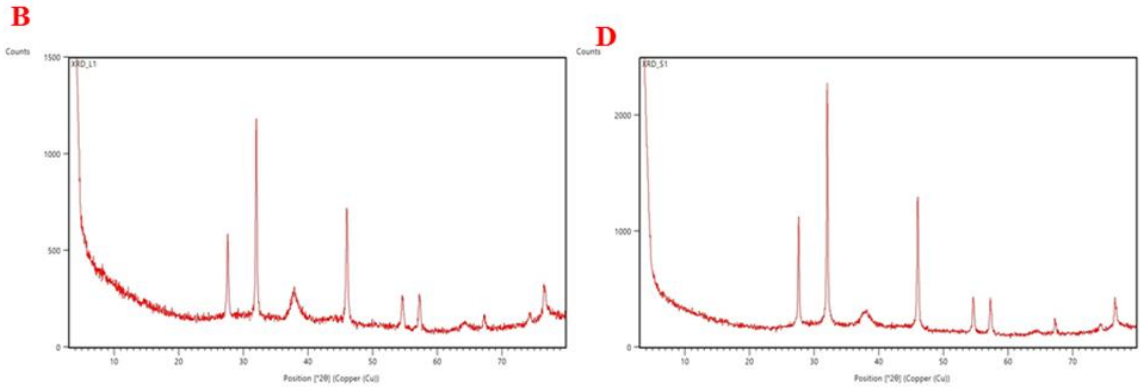


Fig. 2: AgNPs Characterization (A), FT-IR spectra of AgNPs-L (B) X-ray diffraction (XRD) pattern of AgNPs-L (C) FT-IR spectra of AgNPs-S (D) X-ray diffraction (XRD) pattern of obtained from AgNPs-S.

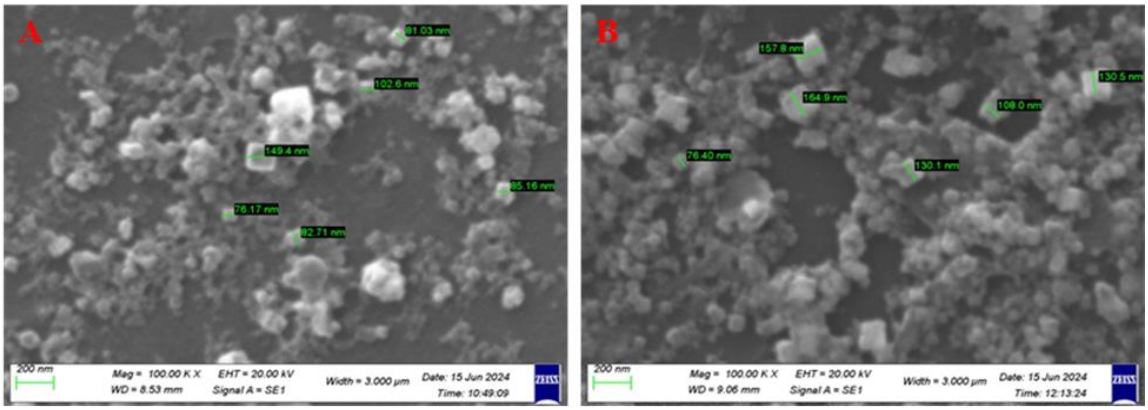


Fig. 3: AgNPs Characterization (A) SEM image of AgNPs-L(B) SEM image of AgNPs-S.

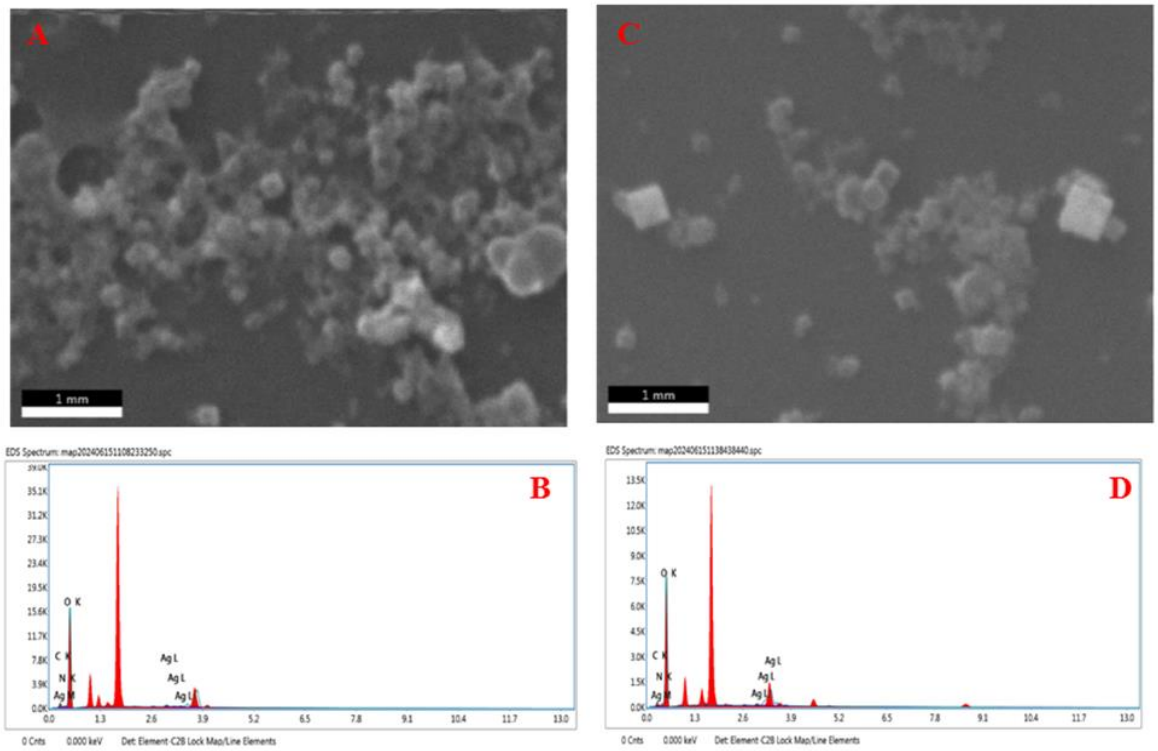


Fig. 4: SEM-EDAX pattern for synthesized AgNPs (A, B) elemental analysis of AgNPs-L (C, D) elemental analysis of AgNPs-S.

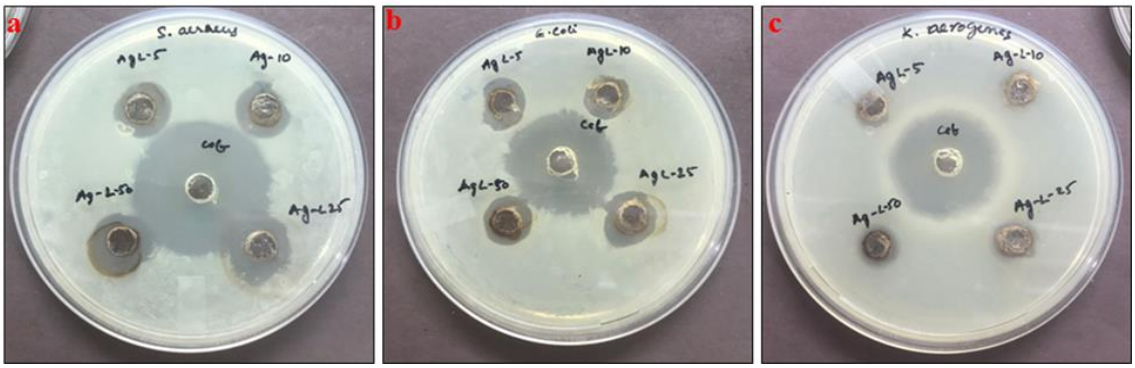


Fig. 5A: Antimicrobial activity of AgNPs-L against various pathogenic bacterial strains shown by Agar well diffusion method (a) *S. aureus* MTCC-96; (b) *E. coli* MTCC-443; (c) *K.aerogenes* MTCC-111.

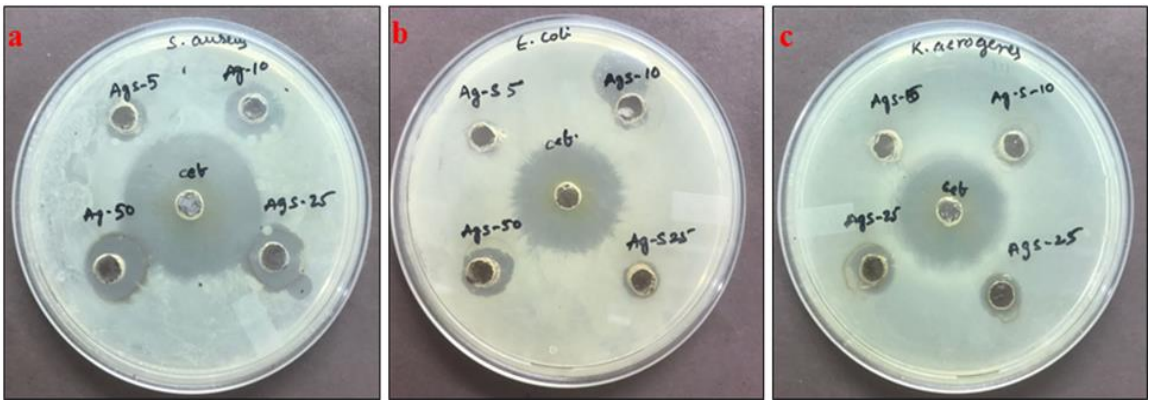


Fig. 5B: Antimicrobial activity of AgNPs-S against various pathogenic bacterial strains shown by Agar well diffusion method (a) *S. aureus* MTCC-96; (b) *E. coli* MTCC-443; (c) *K.aerogenes* MTCC-111.

CONCLUSIONS

Plant-based nanoparticles offer an important alternative to chemical and physical approaches for idiomatic expression. AgNPs were successfully produced using both leaves and seed extracts of *C. paniculatus*. This work employed various characterization techniques, namely UV-spectra analysis, FTIR analysis and XRD method, and SEM analysis, to confirm the presence of AgNPs. The AgNPs produced from plant leaf extract have significant antibacterial activity, making them a promising candidate for application in the field of nanomedicine. The study proven that using the leaves and seeds of *C. paniculatus* to produce AgNPs, In addition, this method is very simple, rapid, and natural, as it does not necessitate any specific tools or procedures for synthesis. Therefore, we can investigate it within the field of medicine.

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