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COMPARATIVE ANTIMICROBIAL EFFICACY OF GREEN-SYNTHESISED SILVER NANOPARTICLES DERIVED FROM MEDICINAL PLANTS OF ACHANAKMAR BIOSPHERE RESERVE, CHHATTISGARH, INDIA

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ABSTRACT

The environmentally friendly, cost-effective, and biologically relevant green synthesis of metal nanoparticles has recently arisen as a viable substitute for traditional chemical methods. In the present study, silver nanoparticles (AgNPs) were biosynthesised using methanolic leaf extracts of three medicinal plants, namely *Bauhinia purpurea*, *Buchanania lanzan*, and *Boerhavia diffusa*, collected from the Achanakmar Biosphere Reserve, Chhattisgarh, India. The plant extracts' phytochemicals served as organic stabilising and reducing agents, allowing the creation of nanoparticles without the use of dangerous chemicals. The prepared AgNPs were studied by means of X-ray diffraction, scanning electron microscopy, energy dispersive X-ray spectroscopy, scanning electron microscopy, ultraviolet-visible spectroscopy, and Fourier transform infrared spectroscopy to ascertain their crystallinity, size, composition of elements, and formation. The nanoparticles predominantly exhibited spherical morphology with nanoscale dimensions and good crystallinity. Using the agar well diffusion method, antimicrobial activity was evaluated against *Escherichia coli* and *Candida albicans* using the agar well diffusion method. *Boerhavia diffusa*-mediated AgNPs demonstrated the strongest antibacterial activity among the synthesised nanoparticles, whereas *Buchanania lanzan*-derived AgNPs demonstrated enhanced antifungal potential. The findings highlight the influence of phytochemical diversity on nanoparticle characteristics and biological performance. This study contributes to the growing field of green nanotechnology by demonstrating the potential of medicinal plants from the Achanakmar Biosphere Reserve as sustainable resources for the development of biologically active silver nanoparticles with promising antimicrobial and Therapeutic use.

Keywords: Nanotechnology, Bio Synthesis, Medicinal Plants, Antimicrobial Activity, *Escherichia Coli, Candida Albicans*

RASĀYAN *J. Chem.*, Vol. 19, No.3, 2026

INTRODUCTION

Nanotechnology has emerged as a rapidly advancing field owing to the unique physicochemical properties of nanoparticles at the nanoscale level (1 to 100 nanometers).[1] Since they are relatively small and have a high surface-to-volume ratio, they possess distinctive chemical, biological, and structural characteristics that are very different from those of the larger quantities.[2] Silver nanoparticles, also known as AgNPs, have attracted a lot of attention among metallic nanoparticles due to the exceptional optical, catalytic, and antibacterial capabilities that they possess. Silver nanoparticles, also known as AgNPs, have attracted considerable attention among metallic nanoparticles due to their exceptional optical, catalytic, and antibacterial properties. Traditional synthesis methods frequently utilise hazardous chemicals and energy-intensive procedures that present environmental risks.[3] Green synthesis technologies that utilise biological resources like plant extracts have been developed in response to the need for environmentally friendly substitutes.[4] According to recent research, plant-mediated synthesis of silver nanoparticles improves biocompatibility and antimicrobial efficiency while offering a sustainable, economical, and environmentally safe substitute for traditional chemical synthesis techniques.[5] Since their phytochemicals, flavonoids, alkaloids, phenols, terpenoids, and reducing sugars act as reducing and stabilising factors and

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eliminate the need for external chemical reagents, plants are particularly advantageous for the creation of nanoparticles.[6] In addition to aiding in the reduction of silver ions, phytochemicals like flavonoids, phenolics, alkaloids, terpenoids, and reducing sugars have a major impact on the morphology, stability, crystallinity, and biological activity of the produced nanoparticles.[5] Green-synthesised AgNPs have demonstrated remarkable antimicrobial activity against multidrug-resistant bacterial and fungal pathogens, suggesting their potential application in biomedical and pharmaceutical fields.[7] While there have been reports of medicinal plant-based green synthesis of silver nanoparticles, comparative studies involving medicinal flora from the Achanakmar Biosphere Reserve are severely inadequate. Furthermore, the influence of phytochemical diversity among these medicinal plants on the antimicrobial efficacy of biosynthesised AgNPs has not been systematically evaluated. Therefore, the present study attempts to address this research gap by comparatively investigating silver nanoparticles synthesised from selected medicinal plants of this ecologically important region.[8] This study's investigation of environmentally friendly nanomaterials with possible antibacterial uses is strongly related to Sustainable Development Goal 3 (Good Health and Well-Being). By reducing the environmental impact of traditional chemical synthesis methods, the creation of plant-mediated silver nanoparticles may help develop sustainable biomedical solutions to fight microbial diseases. This research examines three specific medicinal plants: *Bauhinia purpurea*, *Buchanania lanzan*, and *Boerhavia diffusa*. Each plant is recognised for its bioactive substances that possess antibacterial and antioxidant properties. Nevertheless, their combined potential for the biosynthesis of AgNPs remains unexamined. Therefore, the present study aims to synthesise green silver nanoparticles using selected medicinal plants from the Achanakmar Biosphere Reserve, characterise the synthesised nanoparticles using advanced analytical techniques, and comparatively evaluate their antimicrobial efficacy against selected microbial strains.

EXPERIMENTAL

Taking samples of plant material

Nutritious and fresh leaves of *Bauhinia purpurea L.* (Kachnar), *Buchanania lanzan Spreng.* (Chironji), and *Boerhavia diffusa L.* (Punarnava) were collected from the Achanakmar Biosphere Reserve, Mungeli District, Chhattisgarh, India. The collecting site is located at roughly longitude 81.76° E and latitude 22.36° N, situated within the tropical deciduous forest region of central India. The gathered leaves had been meticulously sterilised with standard distilled water, allowed to air-dry for ten to fifteen days at ambient temperature in the shade, and subsequently pulverised and ground into a fine powder in a sterilised grinder.

Fig.-1: Images of Leaves of Medicinal Plants used (a) *Bauhinia purpurea*, (b) *Buchanania lanzan*, and (c) *Boerhavia diffusa*

Verification of Botanical Specimens

The plant sample was confirmed by representatives of the Botanical Survey of India (BSI), the Central Regional Centre in Prayagraj (Allahabad), and the Indian Ministry of Environment, Forest, and Climate Change. Dr Vinay Ranjan, Head of Office and Scientist-E at BSI Prayagraj, performed the authentication. (Ref. No. CRC/TECH/2025-26/557).

Preparation of Methanolic Extracts

100 grams of dried leaf powder from each plant was subjected to methanol extraction using a Soxhlet apparatus. Extraction was continued for 48 hours at 60 °C until the siphon solvent became clear. The extracts were concentrated by evaporating methanol in a bath of water at about 50 °C to remove excess

solvent. The viscous, semisolid extracts were collected in sterile glass jars and stored at 4 °C for future use in nanoparticle manufacturing.[9,10]

Forming AgNPs Biochemically

A newly prepared aqueous solution of silver nitrate ($AgNO_3$) with a concentration of 1 mM was utilised as the precursor. A conical flask of 250 mL was utilised for each plant, containing 10 millilitres of an extract made from methanol and 90 milliliters of 1 mM $AgNO_3$. After that, at 25°C room temperature, the mixture was stirred for 1.5 hours using a magnet. The creation of silver nanoparticles (AgNPs) and the transformation of silver ions into silver metal were both communicated through the use of a colour gradient that ranged from bright yellow to dark brown. Three unique samples were synthesised: BD-AgNP from *Boerhavia diffusa*, BP-AgNP from *Bauhinia purpurea*, and BL-AgNP from *Buchanania lanzan*. Upon completion of the process, each liquid was chilled and subsequently centrifuged at 10,000 revolutions per minute for fifteen minutes at a time. To remove any unbound phytochemicals, these pellets were washed twice with distilled water and methanol. After that, for a period of three hours, they underwent drying in a combustion oven with temperatures ranging from fifty to sixty degrees Celsius. These unadulterated nanoparticles have been preserved in hermetically sealed vials for characterisation and bioassays.

Synthesised AgNPs Characterisation

The produced AgNPs were examined using many instrumental techniques to verify their synthesis, surface chemistry, and crystalline structure. Using ultraviolet-visible spectroscopy (UV-Vis), optical absorption spectra were obtained in the 300-700 nm range. A distinct peak in the 400-450 nm range of Surface Plasmon Resonance (SPR) was used to confirm the presence of nanoparticles. This study shows that phytochemicals can stabilise and reduce AgNPs at specific Fourier Transform Infrared Spectroscopy (FTIR) wavelengths, specifically C-N, C=O, and -OH. The crystalline structure with characteristic peaks at 2θ signifying 38°, 44°, 64°, and 77° was revealed by the results of XRD examinations, which proved the presence of Face-Centred Cubic (FCC) AgNPs.[13] The pureness of the nanoparticles, as well as their elemental composition were validated by the use of Energy-Dispersive X-ray (EDX) analysis, while Scanning Electron Microscopy (SEM) recorded the surface shape and size distribution.[14] Nanoparticles' uniform distribution and nanoscale properties were confirmed by analysing TEM (Transmission Electron Microscopy) pictures, which revealed their shape and average size.[15]

Antimicrobial Assay

In order to determine whether the AgNPs were efficient, the standard agar-based diffusion well technique was utilised for testing them against two bacterial strains: *Escherichia coli*, a bacterium that is Gram-negative, and *Candida albicans*, a type of fungi. Wells measuring 6 mm in diameter were filled with 100 microliter (μL) each of the AgNP suspension after the nutritional agar and Sabouraud dextrose agar plates were prepared. The control group continued to be free of nanoparticles. An incubation period of one day at 37 °C allowed for the measurement of inhibitory zones in millimetres from the specimens. Had run three separate experiments to ensure accuracy.[16]

RESULTS AND DISCUSSION

Analysing the Production of AgNPs Visually

As a visual indicator of the synthesis of AgNPs, the reaction mixture's colour changed from a pale yellow to a deeper brown after being incubated with plant extracts. The colour change was brought about by the surface plasmon resonance (SPR) of AgNPs.

Synthesised AgNPs Characterisation

Analysis from Ultraviolet-Visible Spectroscopy

The ultraviolet visible spectrum from the produced nanoparticles of silver showed clear absorption bands from surface plasmon resonance, measuring between 414 and 425 nm, which confirmed the synthesis of AgNPs. A significant reduction in transmittance and a concomitant rise in absorbance in this region signified pronounced Surface Plasmon Resonance and electron oscillation. Punarnava (BD-AgNP) exhibited a smooth peak at 420 nm, Kachnar (BP-AgNP) had a sharp and intense peak at 425 nm, and Chironji (BL-AgNP) presented a larger peak at 414 nm.

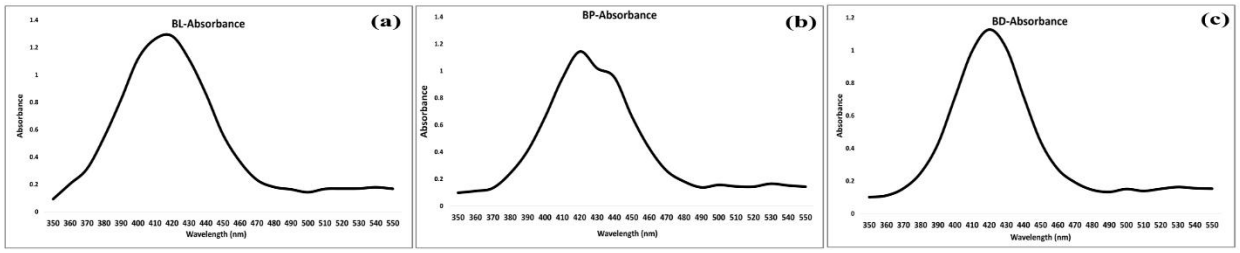

Fig.-2: Biosynthesised AgNPs UV-Visible Absorbance Spectra with SPR Bands: (a) *Buchanania lanzan* AgNPs, (b) *Bauhinia purpurea* AgNPs, and (c) *Boerhavia diffusa* AgNPs

Analysis Utilising FTIR or Fourier Transform Infrared Spectroscopy

FTIR verified that proteins generated from plants affected the stability and reduction of the biosynthesised AgNPs. The O-H stretching vibrations of phenolic compounds were indicated by a wide absorption spectrum in all samples, which occurred between 3300 and 3440 cm^{-1}. The presence of proteins and polyphenols in nanoparticle capping is suggested by observable peaks between 1620-1635 cm^{-1}, which are ascribed to the C=O stretch of amide or flavonoid groups. The bands seen between 1050-1380 cm^{-1} represent vibrations of stretching caused by the C-O as well as bonds composed of C-N associated with plant extracts' alcohols, polysaccharides, and flavonoids. The creation of nanoparticles of silver is confirmed by the appearance of low-frequency bands between 500-600 cm^{-1}, which are attributed to Ag-O vibrations. Minor shifts in the zenith angles of *Buchanania lanzan*, *Bauhinia purpurea*, and *Boerhavia diffusa* suggest differences in phytochemical content that affect nanoparticle stabilisation.

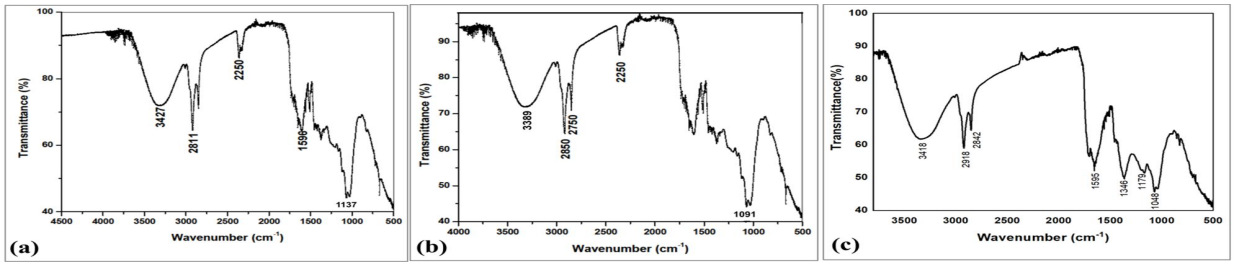

Fig.-3: Biosynthesised AgNPs FTIR Spectra Showing Functional Groups (a) *Buchanania lanzan* AgNPs, (b) *Bauhinia purpurea* AgNPs, and (c) *Boerhavia diffusa* AgNPs

Analysis by X-Ray Diffraction

The crystalline structure of the synthesised AgNPs was confirmed by X-ray diffraction analysis. Peaks at 2θ values near 38°, 44°, 64°, and 77° were clearly visible in the diffraction patterns of all the samples, as is expected for JCPDS data. Metallic silver's (111), (200), (220), and (311) planes are matched by these peaks. Here, crisp and powerful reflections signify the high crystallisation of the nanoparticles, while the lack of further impurity peaks verifies their phase purity, ruling out oxide formation.

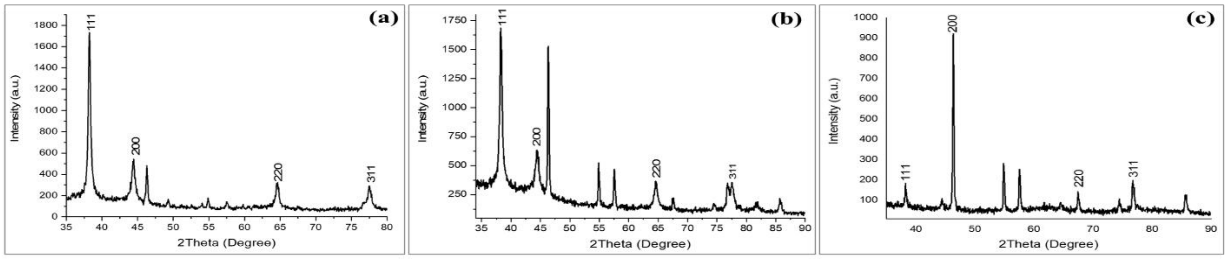

Fig.-4: Biosynthesised AgNPs X-ray Diffraction Patterns: (a) *Buchanania lanzan* AgNPs, (b) *Bauhinia purpurea* AgNPs, and (c) *Boerhavia diffusa* AgNPs

Subtle discrepancies in peak strength and broadening across the samples indicate changes in crystallite size and crystallinity, attributable to differences in the phytochemical makeup of the plant extracts. The XRD data confirm the successful green production of crystalline AgNPs.

Analysis using SEM and EDX

Scanning Electron Microscopy identified AgNPs that were mostly spherical to quasi-spherical in form. These nanoparticles had a moderate amount of aggregation and had a surface shape that was rather smooth. Nanoparticles exhibited a rather uniform distribution, with sizes predominantly within the nanometer scale, signifying effective production by plant-mediated synthesis. The phytochemical composition of the plant extracts may explain the slight differences in particle size and surface texture across samples. The produced nanoparticles were confirmed to have a predominantly silver elemental composition by energy-dispersive X-ray analysis. Additionally, carbon and oxygen signals from plant-derived capping agents were also present. Trace elements, including chlorine, potassium, silicon, and sulfur, are derived from the naturally existing constituents of the plant extracts. The SEM and EDX data collectively validate the synthesis of plant-capped AgNPs exhibiting stable shape and good elemental purity.

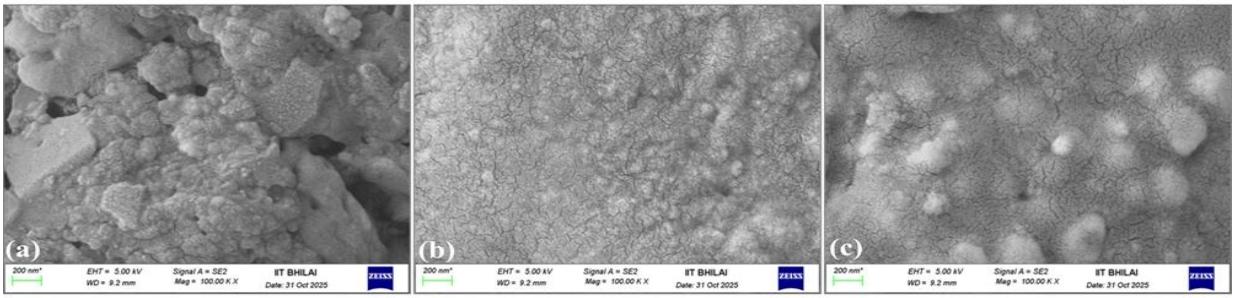

Fig.-5: Biosynthesised AgNPs SEM Micrographs Showing Surface Morphology: (a) *Buchanania lanzan* AgNPs, (b) *Bauhinia purpurea* AgNPs, and (c) *Boerhavia diffusa* AgNPs

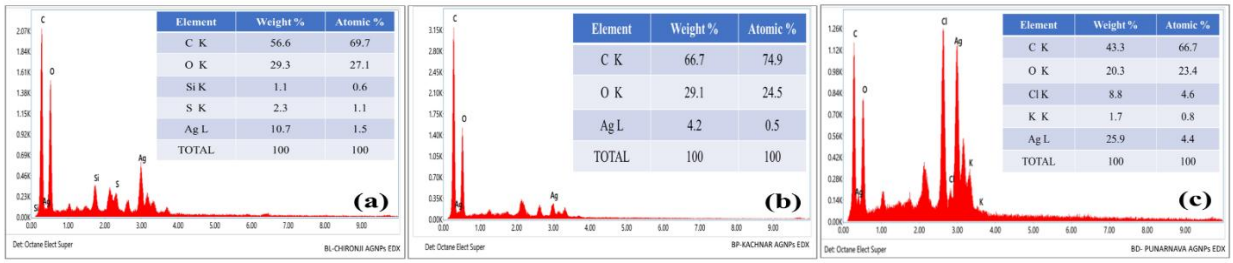

Fig.-6: Biosynthesised AgNPs EDX Spectra Confirming the Presence of Elemental Silver and Associated Surface-Bound Biomolecules: (a) *Buchanania lanzan* AgNPs, (b) *Bauhinia purpurea* AgNPs, and (c) *Boerhavia diffusa* AgNPs

Image Analysis with A Transmission Electron Microscope

The obtained AgNPs were confirmed to be crystalline, evenly distributed, and mostly spherical using transmission electron microscopy analysis. The nanoparticles exhibited homogeneous distribution within the nanometer scale, with distinct lattice fringes evident, signifying high crystallinity.

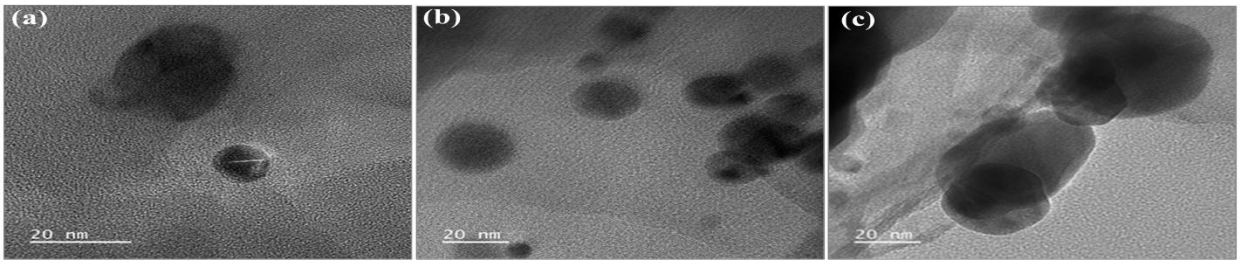

Fig.-7: Biosynthesised AgNPs TEM Images Showing Spherical Morphology, Uniform Dispersion, and Crystalline Structure: (a) *Buchanania lanzan* AgNPs, (b) *Bauhinia purpurea* AgNPs, and (c) *Boerhavia diffusa* AgNPs

The slight variations in particle size across the samples suggest that the plant extracts used for the synthesis have changed their phytochemical characteristics. The results of the TEM verify the

morphological as well as crystalline properties that were detected in the SEM and XRD studies, hence confirming the efficient and sustainable manufacturing of AgNPs.

Antimicrobial Efficacy

The biological effectiveness of the produced AgNPs was demonstrated by the presence of significant inhibitory zones against *Escherichia coli* and *Candida albicans*, which have been identified through antimicrobial assays.

Table-1: Comparing the Antimicrobial Activity of *Buchanania lanzan*, *Bauhinia purpurea*, and *Boerhavia diffusa*-AgNPs against *Candida albicans* and *Escherichia coli*

Microorganism	*Boerhavia diffusa* AgNPs *(Punarnava)*	*Bauhinia purpurea* AgNPs *(Kachnar)*	*Buchanania lanzan* AgNPs *(Chironji)*	Control ($AgNO_3$ / Blank)
Escherichia coli	+++ (Good inhibition)	++ (Moderate inhibition)	- (No inhibition)	-
Candida albicans	- (No inhibition)	- (No inhibition)	++ (Fungal inhibition)	-

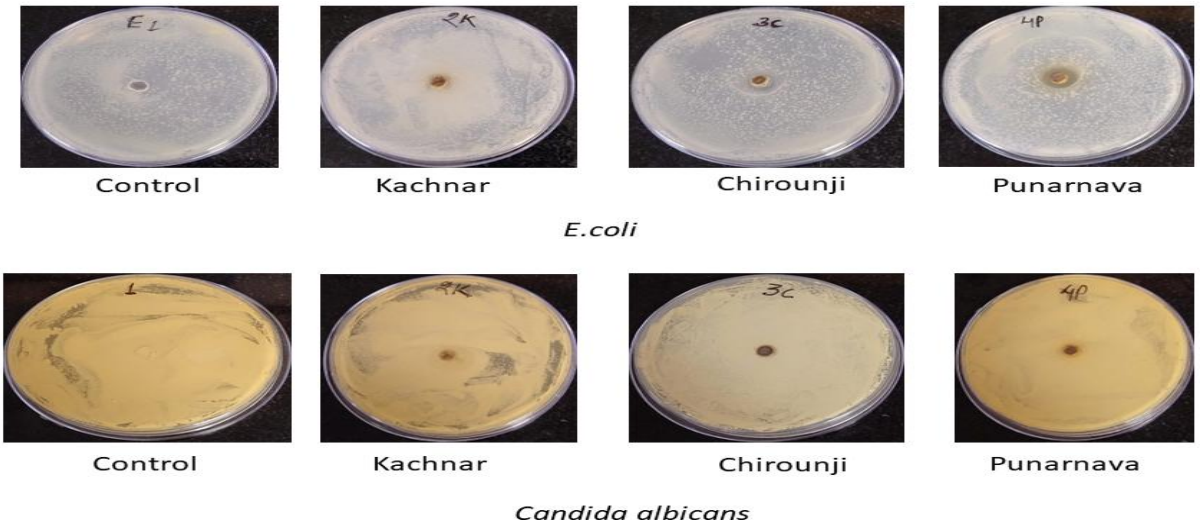

Fig.-8: Agar Well Diffusion Assay Showing Inhibition Zones for *Buchanania lanzan*, *Bauhinia purpurea*, and *Boerhavia diffusa*-AgNPs

The present work demonstrated that AgNPs may be greenly synthesised utilising methanolic leaf extracts of Achanakmar Biosphere Reserve plants, specifically *Boerhavia diffusa*, *Bauhinia purpurea*, and *Buchanania lanzan*. The produced nanoparticles demonstrated a crystalline structure, spherical shape, and nanoscale dimensions, as verified by spectroscopic and microscopic investigations. Among the analysed samples, nanoparticles generated from *Boerhavia diffusa* exhibited superior antibacterial efficacy, while those from *Buchanania lanzan* displayed significant antifungal activity, underscoring the impact of the phytochemical formula on nanoparticle bioactivity. The findings underline the potential of AgNPs derived from medicinal plants as eco-friendly antibacterial agents and support further research in biological and ecological settings. The antimicrobial potential and environmentally sustainable synthesis approach of the present study also support Sustainable Development Goal 3 (Good Health and Well-Being) by encouraging the development of eco-friendly nanomaterials for biomedical and antimicrobial applications.

CONCLUSION

The current research was able to successfully demonstrate the green synthesis of silver nanoparticles (AgNPs) by utilising methanolic leaf extracts of *Boerhavia diffusa*, *Bauhinia purpurea*, and *Buchanania lanzan* that were collected from the Achanakmar Biosphere Reserve. Spectroscopic and microscopic analyses confirmed the crystalline nature, spherical morphology, and nanoscale dimensions of the synthesised nanoparticles. Among the investigated samples, *Boerhavia diffusa*-mediated AgNPs exhibited

superior antibacterial activity, whereas *Buchanania lanzan*-derived AgNPs showed significant antifungal efficacy, indicating the important role of phytochemical composition in determining nanoparticle bioactivity. The findings highlight the potential of medicinal plant-mediated AgNPs as eco-friendly antimicrobial agents with promising biomedical applications. Further investigations involving toxicity assessment and in vivo studies are recommended to explore their practical therapeutic potential. The present work also supports Sustainable Development Goal 3 (Good Health and Well-Being) by promoting the development of environmentally sustainable nanomaterials for antimicrobial and healthcare applications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-9725/2026]