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BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING LEAVES EXTRACT OF *Zanthoxylum armatum* AND THEIR ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL

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ABSTRACT

Plant-mediated nanoparticle synthesis offers several advantages over conventional methods, including reduced toxicity, enhanced stability, sustainability, and cost-effectiveness. This study focused on the synthesis of silver nanoparticles(ZA-AgNPs) using the leaf extract of *Zanthoxylum armatum*(ZAE). The ZA-AgNPs were characterized using various advanced analytical techniques. UV-visible spectroscopy revealed a peak at 419 nm. DLS analysis indicated an average Z-size of 159 nm, a polydispersity index (PDI) of 0.343, and a zeta potential of -16.3 mV, presenting a single peak. FE-SEM images showed spherical AgNPs within a size range of 30-50 nm. FTIR analysis identified functional groups, displaying peaks within the range of 3437-600 cm^{-1}, while XRD confirmed the crystalline nature of the nanoparticles. The antimicrobial susceptibility of the silver nanoparticles was assessed against eight bacterial and six fungal strains. The ZA-AgNPs exhibited significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* at concentrations of 10-20 µg/mL. Additionally, they demonstrated potent antifungal activity against *Fusarium oxysporum* and *Aspergillus niger* at a concentration of 50 µg/mL. Furthermore, the ZA-AgNPs displayed robust antioxidant activity in comparison to the ZAE in DPPH, RPA, and TAC assays. In conclusion, the study supports the eco-friendly synthesis of AgNPs from *Z. armatum* and highlights their antimicrobial and antioxidant properties.

Keywords: Biogenic Synthesis, *Zanthoxylum Armatum*, Silver Nanoparticles, Antimicrobial, Antioxidant.
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INTRODUCTION

Human health is threatened by rising antibiotic-resistant microbes, overuse of antimicrobials, and toxic free radicals.[1,2] Efforts have focused on developing novel, efficient, and eco-friendly solutions, such as biogenic nanoparticles, to address these challenges.[3] Nanobiotechnology applies green chemistry to develop sustainable nanomaterial synthesis and design nanoscale products with efficient energy use, minimal waste, and enhanced safety.[4] This interdisciplinary field has significant potential in biotechnology, medicine, environmental engineering, microbial resistance, and plant protection.[5] Nanoparticles can be synthesised using physical, chemical, or biological methods.[6,7] Biomimetic synthesis is preferred for its low toxicity, cost-effectiveness, energy efficiency, and environmental sustainability.[8] Green synthesis, using microbial enzymes or plant phytochemicals with antioxidant or reducing properties, is commonly used to convert metallic compounds into nanoparticles.[3,9] Currently, researchers are investigating how different medicinal plants can be used to produce nanoparticles to improve the efficiency of plant metabolites.[10] Plant extracts contain various metabolites that are crucial for reducing and stabilising nanomaterials, acting as both capping and reducing agents.[11] Nanoparticles have been synthesised from various metals, including zinc, silver, gold, copper, iron, nickel, and titanium.[12] Silver nanoparticles are particularly noteworthy because of their dispersion, stability, large surface area, chemical inertness, and unique absorption properties in the visible region, which enhance their biocompatibility.[13] Previous studies indicated that silver nanoparticles (AgNPs) are minimally hazardous to eukaryotic cells but exhibit high toxicity to prokaryotes.[14,15] Biogenically synthesised AgNPs have a range of applications in medicine, including drug delivery, cancer treatment, and possess antimicrobial, antioxidant, and anti-inflammatory properties.[16] Moreover, medicinal plants have historically played a crucial role in human health and serve as a rich source of therapeutic compounds.[17] *Z. armatum*,

commonly called Indian prickly ash[18], is native to the tropical and sub-tropical Himalayas, specifically at altitudes of 1000 to 2500 meters.[19] Its fruits, bark, leaves, stem, and seeds possess various medicinal properties and are used to treat toothaches, aid digestion, promote stomach health, reduce fever, stimulate appetite, and alleviate dyspepsia.[20] It contains various secondary metabolites such as flavonoids, alkaloids, coumarins, lignans, amides, terpenoids, linalool, beta-sitosterol, tamblin, tambulatin, aramatamide, asarinin, and fragesin.[21] These metabolites contribute to numerous pharmacological properties[22], including antidepressant, hepatoprotective[23], anti-diabetic, anti-spasmodic, antinociceptive, antiproliferative, antimicrobial, antioxidative, antiinflammatory, larvicidal, piscicidal, antitumor, and memory-enhancing effects and a soothing effect on skin.[24] *Z. armatum* also shows potential against SARS-CoV-2 and *H. pylori*, and also exhibits anti-urease activity in vitro and in silico.[25] Its compounds make it suitable for enhancing pharmacological properties through the green synthesis of silver nanoparticles.[26] In view of the promising medicinal properties of *Z. armatum*, this study reports the biogenic synthesis of AgNPs using *Z. armatum* leaves extract. The AgNPs were characterized using advanced analytical instruments. Their antioxidant and antimicrobial activities, including minimum inhibitory concentration against bacterial and fungal strains, were also evaluated. *Z. armatum* leaves extract-mediated AgNPs can be used to combat the multidrug-resistant microbes.

EXPERIMENTAL

Materials Used

Methanol, dimethyl sulfoxide, nutrient agar, potato dextrose agar, streptomycin, chloramphenicol, ciprofloxacin, gentamicin, fluconazole, ketoconazole, DPPH (2,2-Diphenyl-1-picrylhydrazyl), phosphate buffer, sulphuric acid, ascorbic acid, silver nitrate, sodium phosphate (monobasic), sodium phosphate (dibasic), ammonium molybdate, ferric chloride, potassium ferricyanide, trichloroacetic acid were purchased from SRL, Himedia and Sigma, India. All working solutions were freshly prepared in double-distilled water and sterilised by autoclaving as needed.

Collection and Processing of Samples

Fresh and healthy leaves of *Z. armatum* were collected from naturally growing plants in the Ramban forest region of Jammu and Kashmir, India. The collected samples were washed thoroughly with tap water and distilled water and then dried in the shade until completely dry before extraction.

Preparation of Plant Extract

The crude leaf extract of *Z. armatum* was prepared.[27] Briefly, 10 g of powdered dried leaves was mixed with 100 mL of methanol and incubated at 40° C for seven days with shaking at 150 rpm. The filtrate was subsequently evaporated in a fume hood until dryness, and the resulting residue was dissolved in DMSO at a concentration of 1mg/mL for the synthesis of silver nanoparticles.

Synthesis of Silver Nanoparticles

To synthesise AgNPs, 80 mL of 1 mM $AgNO_3$ solution was stirred for 15 minutes before adding 20 mL of crude leaves extract (1g/mL). The mixture was stirred at 615 rpm and 73° C until the colour changed from dark green to dark brown, and then left overnight under the same conditions. The colour change gives initial confirmation for AgNPs synthesis. The solution was centrifuged at 8,000 rpm for 20 minutes, then rinsed with double-distilled water and ethanol, and air-dried in a fume hood. The resulting pellet was used for further analysis.[11]

Characterization

Biogenically synthesised AgNPs were characterised using advanced analytical techniques. UV-Vis spectroscopy (UV-1800 Shimadzu) was used to measure the optical density of AgNPs.[28] Dynamic light scattering (Malvern Instruments) was used to assess average size and zeta potential.[29] Fourier transform infrared spectroscopy (FTIR, Spectrum Two/Perkin Elmer) identified functional groups in the range 4000-400 cm^{-1}.[30] Field emission scanning electron microscopy (SEM, JSM-7610FPlus, JEOL) was used to assess the surface morphology of AgNPs.[31] X-ray diffraction (XRD, Smart Lab 3KW/Rigaku) was used to analyse the crystalline structure using a monochromatic Cu Kα1 radiation (λ = 0.154 nm) at 2θ with 50 kV and 40 mA.[32]

Antimicrobial Assay

The antimicrobial activity of AgNPs (1 mg/mL) and crude plant extract (1 mg/mL) was evaluated against eight pathogenic bacterial strains: *Streptococcus gordonii* (MTCC-2695), *Bacillus subtilis* (MTCC-421), *Staphylococcus aureus* (MTCC-3160)*, Pseudomonas aeruginosa* (MTCC-647), *Pseudomonas fluorescens* (MTCC-664), *Escherichia coli* (MTCC-16521), *Ralstonia eutropha* (MTCC-8320), *Salmonella enterica* (MTCC-660), and six pathogenic fungal strains: *Fusarium oxysporum* (MTCC-10270), *Alternaria solani* (MTCC-2101), *Rhizopus oryzae* (MTCC-262), *Aspergillus flavus* (MTCC-13062), *Aspergillus fumigatus* (MTCC-2508), and *Aspergillus niger* (MTCC-281), following the agar well diffusion method.[33] Fresh bacterial and fungal cultures (100 µL each) were spread on nutrient agar (NA) and potato dextrose agar (PDA) plates, respectively. The inoculated plates were maintained under incubation at 37°C for bacteria and 28°C for fungi for 12–24 hours. After incubation, four wells were bored in each plate and loaded with 100 µL of positive control (standard drug), negative control (DMSO), ZAE, and ZA-AgNPs. The plates were subsequently incubated overnight at 37°C for bacteria and 28°C for fungi. The zones of inhibition (ZOI) were measured to assess antimicrobial activity. The minimum inhibitory concentration (MIC) is the minimum concentration of plant extracts, or AgNPs, required to inhibit microbial growth. The MICs of ZA-AgNPs and ZAE were determined against the specified pathogens.[34]

Antioxidant Activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The DPPH free radical scavenging activity of ZAE and AgNPs was assessed according to a previously described method.[35] A reaction mixture was prepared separately containing concentrations of the crude extract and AgNPs ranging from 20 to 200µg/mL. Subsequently, 1 mL of 0.1 mM methanolic DPPH solution was added, and the mixture was incubated for 30 minutes at room temperature in the dark. The optical density was measured at 517 nm, and lower absorbance indicated a higher level of free radical inhibition. The experiment was conducted in triplicate, using ascorbic acid utilized as a standard. The inhibition percentage of DPPH was calculated using the following formula:

$$\text{DPPH inhibition (\%)} = (Ac - As)/Ac \times 100$$

Where, Ac - absorbance of the control, As- absorbance of the sample.

Reducing Power Assay (RPA)

The reducing power of ZAE and AgNPs was assessed[36], with minor modifications. A 100 µL aliquot of each sample, ranging from 20 to 200 µg/mL, was combined with 250 µL of sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was then incubated at 50°C for 25 minutes. Following the addition of 250 µL of 10% trichloroacetic acid, the mixture was centrifuged at 3600 rpm for 10 to 15 minutes. The supernatant (250 µL) was then mixed with an equal volume of distilled water and 0.1% ferric chloride. After further incubation for 10 minutes, 200 µL of the sample was transferred to an ELISA plate, and the absorbance was recorded at 700 nm. Ascorbic acid served as the standard reference.

Total Antioxidant Capacity / Phosphomolybdate Assay

The phosphomolybdate assay was performed[37], with slight modifications. Different concentrations (20–200 µg/mL) of both ZAE and AgNPs were diluted in DMSO. A 100 µL of each concentration was mixed with equal volumes of sulphuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM) in separate centrifuge tubes. The mixtures were then incubated at 95°C for 95 minutes and cooled to room temperature for 15 minutes. The absorbance was determined at 695 nm. Ascorbic acid was used as a reference. Antioxidant potential was calculated using the provided formula:

$$\text{TAC capacity (\%)} = (Ac - As)/Ac \times 100$$

Where, Ac - absorbance of the control, As- absorbance of the sample

Statistical Analysis

All experiments were performed in triplicate to ensure accuracy, and the obtained results were analysed using one-way analysis of variance (ANOVA) with SPSS statistical software. Statistical significance was determined based on a p-value threshold (<0.05). IC_{50} was determined by linear regression analysis using Microsoft Excel.

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

The crude extract was mixed with an aqueous silver nitrate solution under controlled conditions. Following the addition of the extract, a colour change from light green to dark brown, providing an initial confirmation (Fig.-1 (a)). This colour shift, attributed to bioactive compounds acting as reduction and capping agents, indicates the formation of silver nanoparticles.[38] These findings align with previous reports on the synthesis of AgNPs using extracts from *Lycium shawii* leaves by Kaur *et al.* (2024) and AgNPs derived from citrus fruit juice.[39]

Characterisation of Nanoparticles

UV Visible Spectrophotometer

Silver nanoparticle synthesis was confirmed using a UV-Vis spectrophotometer, which measures the reduction of metal ions via surface plasmon resonance (SPR), caused by the resonant oscillation of conduction electrons at the interface of nanoparticles exposed to incident light.[40] The UV-Vis spectrum displayed a single strong peak at 419 nm (Fig.-1 (b)), indicating the presence of AgNPs. A similar UV-Vis absorption spectrum was reported in another study, where AgNPs were produced from *Z. armatum* leaves extract.[30]

Dynamic Light Scattering (DLS)

DLS measurements indicated that the synthesised ZA-AgNPs possessed a mean hydrodynamic size of 159 nm (Fig.-2 (a)) and a PDI of 0.343, reflecting a moderate monodispersive nature. Typically, PDI values lie between 0.05 and 0.7; lower values signify monodisperse systems, whereas values exceeding 0.7 suggest a polydisperse nature.[6] The zeta potential of the AgNPs was -16.3 mV (Fig.-2 (b)), suggesting adequate stability due to electrostatic repulsion, which prevents aggregation. Similarly, AgNPs derived from *Urtica diocia* leaves extract showed a PDI of 0.47 along with a zeta potential of -15.5 Mv.[29]

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectroscopy reveals absorption peaks that indicate bond vibrations, facilitating the identification of functional groups in nanoparticles based on their distinctive atomic composition.[40] The FTIR analysis of ZA-AgNPs, as illustrated in Fig.-1(c), highlights the functional groups identified by peak values at 3437, 2923, 2854, 1621, 1396, 1037, and 600 cm^{-1} in the infrared radiation spectrum. The prominent peak at 3437 cm^{-1} signifies the N-H stretching vibration of the amino group and the O-H group of phenols. The smaller peaks at 2923, 2854, and 1396 cm^{-1} are associated with C-H stretching in hydrocarbons (alkanes).[41] A sharp peak at 1621 cm^{-1} corresponds to the N-H bond, attributed to the amide I bonds found in proteins.[27], while the peak at 1037 cm^{-1} represents C-N stretching in amines.[29] Additionally, signals at 600 cm^{-1} correspond to C-H stretching in alkenes. The significant presence of C–N, C–C, and O–H groups functions as a capping agent, effectively stabilising the nanoparticles.[42] These results are closely aligned with the findings of Jyoti and Singh, (2016) regarding the synthesis of AgNPs using the extract from *Z. armatum* leaves.[30]

Field Emission Scanning Electron Microscope (FE-SEM) Analysis

The morphological characteristics, including the size and shape of the ZA-AgNPs, were analysed through FE-SEM. The Fig.-1 (e) reveals predominantly spherical nanoparticles, measuring between 30 and 50 nm, with both dispersed and aggregated forms present. This observation aligns with the findings of Riaz *et al.* (2022), who reported similar characteristics in AgNPs synthesised from the bark extract of *Z. armatum*, noting a few irregularly shaped particles as well.[27] The aggregation of the nanoparticles into irregular structures can be attributed to the adhesion of the extracts to their surfaces.[41]

X-Ray Diffraction (XRD)

The XRD patterns presented in Fig.-1 (d) reveal distinctive peaks at 2θ values of 36.25°, 46.26°, 57.54°, and 76.82°, which correspond to the crystal planes (111), (200), (220), and (311), respectively. These observations confirm the face-centred cubic (FCC) structure and the crystalline nature of the synthesised AgNPs. The two unassigned peaks (*) may be associated with bioorganic compounds found in the ZA-

AgNPs. Furthermore, Binsalah *et al.* (2022) documented the FCC structure of AgNPs synthesised using ethyl acetate leaves extract from *Urtica dioica.*[29]

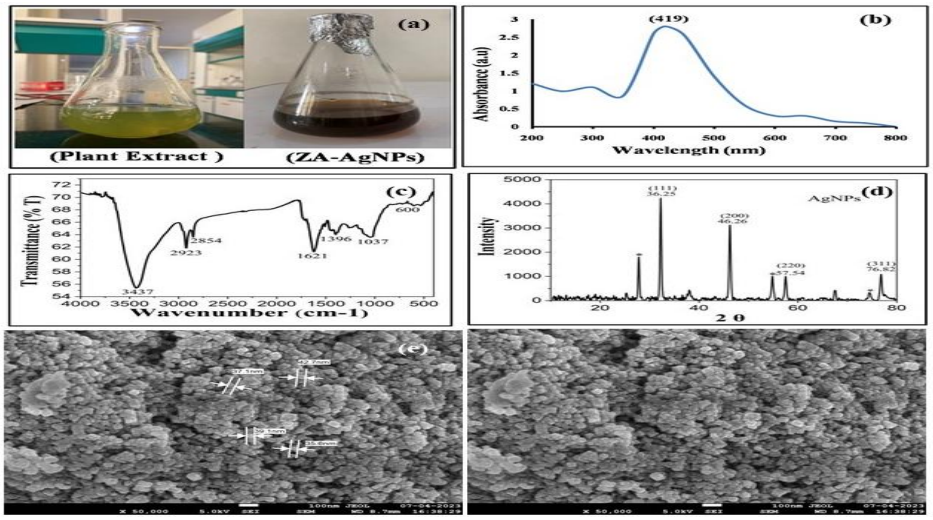

Fig.-1: Biosynthesis of ZA-AgNPs and their Characterisation: (a) Leaves Extract of *Z. armatum* and Silver Nanoparticles (b) UV-visible Spectra of ZA-AgNPs (c) FTIR of ZA-AgNPs (d) XRD of ZA-AgNPs (e) SEM of ZA-AgNPs

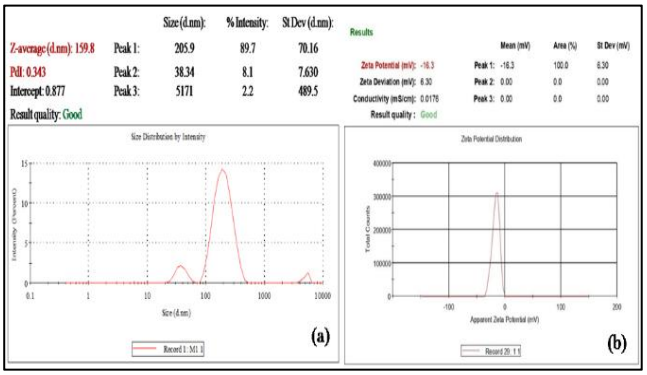

Fig.-2: (a) Dynamic Light Scattering (DLS) and (b) Zeta Potential of ZA-AgNPs

Antimicrobial Assay

Silver nanoparticles, characterised by their small size, stability, and microbicidal potential, are particularly suited for applications in pharmaceuticals, healthcare, the food industry, and water treatment.[43,44] The antibacterial efficacy of both ZAE and ZA-AgNPs was assessed against gram-positive (*B. subtilis*, *S. aureus*, *S. gordonii*) and gram-negative (*P. aeruginosa*, *P. fluorescens*, *S. enterica*, *E. coli*, *R. eutropha*) bacteria, as illustrated in Fig.-3 (a). ZA-AgNPs exhibited the highest zone of inhibition (ZOI) against *B. subtilis* (26.03 ± 0.25 mm) when compared to chloramphenicol (24.03 ± 0.15 mm) and plant extract (20.03 ± 0.35 mm) at a concentration of 50 µg/mL (Table-1), with a significant p-value of <0.001. The ZOI for *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* was greater than that reported for AgNPs derived from *A. sessilis* leaves and oregano root extract.[45] Furthermore, Joshi *et al.* (2024) reported that AgNPs synthesised from the extract of *C. roseus* leaves effectively inhibit *B. subtilis.*[46] The antibacterial mechanism of nanoparticles is influenced by microbial susceptibility. These nanoparticles adhere to bacterial surfaces through electrostatic interactions, and their small size enables them to penetrate via transmembrane proteins and exploit the proton motive force. They preferentially bind to sulfur groups in proteins, leading to the formation of thiols, as well as to phosphates, which can result in DNA damage. The differences in susceptibility between gram-positive and gram-negative strains to AgNPs can be attributed to variations in their cell wall structures.[47]

Table-1: Antibacterial Activity and MIC of Crude Plant Extract and AgNPs of *Z. armatum*

Bacterial Strain	Zone of Inhibition (mm)			MIC (µg/mL)	
	Plant extract	AgNPs	Positive control	Plant extract	AgNPs
B. subtilis	20.03 ± 0.35	26.03 ± 0.25	24.03 ± 0.15	40	20
S. aureus	14.06 ± 0.30	22.03 ± 0.15	30.00 ± 0.40	50	10
S. gordonii	16.00 ± 0.60	23.03 ± 0.35	34.93 ± 0.30	50	10
E. coli	13.06 ± 0.20	20.96 ± 0.25	30.00 ± 0.40	50	30
S. enterica	15.00 ± 0.20	24.96 ± 0.25	44.96 ± 0.45	30	20
P. aeruginosa	15.00 ± 0.10	21.00 ± 0.30	34.00 ± 0.40	50	30
P. fluorescens	16.06 ± 0.30	24.03 ± 0.15	25.96 ± 0.35	50	30
R. eutropha	15.00 ± 0.20	20.03 ± 0.35	31.03 ± 0.15	50	30

Mean ± standard deviation (n = 3); statistically significant ($p < 0.05$)

ZA-AgNPs and ZAE exhibit significant antifungal activity against *A. niger*, *A. fumigatus*, *A. flavus*, *F. oxysporum*, *A. solani*, and *R. oryzae* (Table-2, Fig.-3 (b)). ZA-AgNPs produced ZOI measuring 31.00 ± 0.20 mm for *A. fumigatus*, 28.96 ± 0.35 mm for *A. solani*, and 26.03 ± 0.25 mm for *F. oxysporum*. A similar antifungal efficacy was noted for AgNPs derived from the leaves extract of *C. roseus*.[46] ZA-AgNPs exhibited a larger zone of inhibition (ZOI) against fungi than against bacteria, consistent with findings reported by Zare-Bidaki *et al.* (2022) for AgNPs synthesised from *Medicago sativa* extract.[41] The AgNPs adhered to and saturated the fungal hyphae, destroying fungal cells. The observed inhibitory effect is largely attributed to Ag^+, which disrupts membrane-bound enzymes, especially those involved in cellular respiration.[48]

Table-2: Antifungal Activity and MIC of Crude Plant Extract and AgNPs of *Z. armatum*

Fungal strains	Zone of Inhibition (mm)			MIC (µg/mL)	
	Plant extract	AgNPs	Positive control	Plant extract	AgNPs
A. solani	11.03 ± 0.25	28.96 ± 0.35	19.00 ± 0.30	150	100
F. oxysporum	10.00 ± 0.40	26.03 ± 0.25	11.00 ± 0.10	150	50
A. fumigates	20.00 ± 0.20	31.00 ± 0.30	28.96 ± 0.25	150	75
A. flavus	15.96 ± 0.25	20.00 ± 0.30	16.96 ± 0.35	150	100
A. niger	15.00 ± 0.20	19.00 ± 0.30	15.90 ± 0.60	150	50
R. oryzae	20.10 ± 0.45	25.03 ± 0.35	20.03 ± 0.15	150	100

*** NZ** –No Zone of Inhibition; Mean ± standard deviation (n = 3); statistically significant ($p < 0.05$)

The MIC of ZA-AgNPs and ZAE demonstrated significant efficacy against all bacterial strains tested (Table-1). ZA-AgNPs effectively inhibit gram-positive bacteria at concentrations of 10 to 20 µg/mL and gram-negative bacteria at 50 µg/mL. Specifically, ZA-AgNPs exhibited significant results for *B. subtilis* with an MIC of 20 µg/mL and for *E. coli* at 30 µg/mL, both lower than the MIC observed for the crude plant extract, between 40 and 50 µg/mL. Joshi e*t al.* (2024) reported similar effectiveness, found MICs within the range of 10 to 30 µg/mL for comparable bacterial strains.[46] *S. aureus* and *S. gordonii* were identified as the most susceptible to ZA-AgNPs, with a MIC of 10 µg/mL. This finding is in agreement with Talank et al. (2022), who documented a MIC of 8 µg/mL for AgNPs derived from a seed extract of *Foeniculum vulgare* against *S. aureus*.[49] Furthermore, the MIC of AgNPs was considerably lower than that of the crude extract, aligning with the findings regarding the methanolic fruit extract-based AgNPs of *Aegle marmelosa*.[28] The fungal strains *F. oxysporum* and *A. niger* exhibited the highest susceptibility to ZA-AgNPs, with a MIC of 50 µg/mL. *A. fumigatus* demonstrated susceptibility at 75 µg/mL, whereas *R. oryzae*, *A. flavus*, and *A. solani* showed inhibition at 100 µg/mL (Table-2). Similar investigations into the MIC of AgNPs were carried out for AgNPs against *A. niger*, *A. flavus*, *F. oxysporum*, and *A. fumigatus*.[50] Additionally, Cintesun *et al.* (2025) evaluated the antifungal activity of shilajit extract-based AgNPs against *F. oxysporum* and *F. verticillioides*, noting suppression effects of 65-90% and 50-75%, respectively.[51]

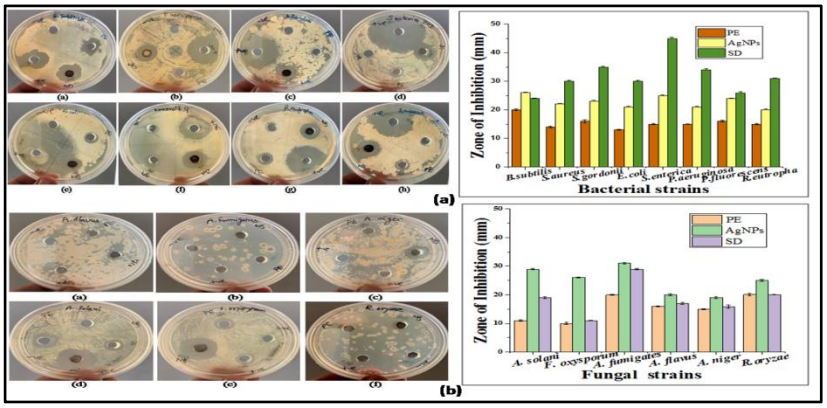

Fig.-3: Antimicrobial Activity (ZOI) of Crude Extract of *Z. armatum*, ZA-AgNPs and Standard Drugs Against Different Bacterial and Fungal Strains: (A) (a) *B. subtilis*, (b) *P. aerugenosa*, (c) *S. gordonii*, (d) *S. enterica*, (e) *E. coli*, (f) *P. fluorescens*, (g) *R. eutropha*, (h) *S. aureus* (B) (a) *A. flavus,* (b) *A. fumigatus,* (c) *A. niger,* (d) *A. solani,* (e) *F. oxysporum,* (f) *R. oryzae*

Antioxidant Activity

Plant secondary compounds exhibit antioxidant properties, which are vital for mitigating diabetes, cancer, oxidative stress, and cardiovascular diseases. The exploration of plant-derived therapeutics highlights their potential as effective and safe treatments owing to their nutraceutical benefits.[52] Their reducing power was evaluated using IC_{50} values; a lower IC_{50} value indicates higher antioxidant or free radical scavenging.[53] AgNPs showed significant antioxidant activity with a p-value < 0.01.

DPPH Assay

The antioxidant activity of *Z. armatum* leaf extract and AgNPs was assessed using the DPPH assay. Both AgNPs and plant extracts donate electrons to DPPH radicals, thereby reducing them to neutral molecules. This reaction results in a colour change from violet to golden yellow, indicating a decrease in radical concentration.[41] The DPPH free-radical scavenging activity increased with concentration (20-200 µg/mL) for both ZAE and ZA-AgNPs, as illustrated in Fig.-5 (a). The free radical scavenging potential of ZAE ranged from 22.52% to 84.19%, whereas ZA-AgNPs exhibited from 59.86% to 93.77%. In comparison, ascorbic acid showed a scavenging potential ranging from 76.41% to 97.77%. The result of this study was better than the previous study, as they possess an antioxidant potential of 82.95% for MeOH-AgNPs derived from *Lycium shawii.*[54] Notably, the AgNPs demonstrated superior radical scavenging activity compared to the plant extract. Sreelekha *et al.* (2021) reported that green-synthesised nanoparticles exhibited 91% scavenging activity, while chemically synthesised counterparts displayed only 79% at the same concentration.[55] The IC_{50} values were determined through a calibration curve, revealing an IC_{50} value for ZA-AgNPs was 47.69 ± 0.002 µg/mL, significantly lower than that of the crude extract (92.11 ± 0.004 µg/mL), yet less effective than ascorbic acid, which had an IC_{50} of 19.26 ± 0.002 µg/mL, as shown in Fig.-5 (d). These findings align with previous results documenting the leaf extract of *Elephantopus scaber.*[56]

Reducing Power Assay

The reducing power activity of ZA-AgNPs and ZAE was evaluated based on their ability to change the colour of the solution from colourless to blue; a more intense blue colour indicates a greater reducing power. Their reduction capacity was quantified using a spectrophotometer set at 700 nm.[57] The dose-response curve (Fig.-5 (b)) demonstrated an increase in a concentration-dependent manner for ZAE, ZA-AgNPs, and ascorbic acid (20–200 µg/mL). The ZA-AgNPs exhibited a high absorbance value of 0.479, compared to ZAE, which had an absorbance of 0.212 at a concentration of 200 µg/mL. In contrast, ascorbic acid achieved an absorbance value of 0.962, indicating that ZA-AgNPs possess greater reducing power than ZAE but less than ascorbic acid. The findings of this study align with the antioxidant potential of AgNPs derived from *Aristolochia bracteolate.*[58] As depicted in Fig.-5(d), the IC_{50} of AgNPs was determined to be 204.36 ± 0.005, the IC_{50} of ZAE was 300 ± 0.001, and the IC_{50} of ascorbic acid was 78.24 ± 0.12 µg/mL. Ascorbic acid was the most effective, followed by AgNPs, with ZAE being the least

effective. Salari et al. (2018) reported that the ability of *Prosopis farcta*-mediated AgNPs to reduce ferric ions to ferrous ions was significantly higher than that of the plant extract at concentrations ranging from 0.2 to 1 mg/mL.[59]

Total Antioxidant Capacity / Phosphomolybdate Assay

The phosphomolybdate method was used to assess the total antioxidant capacity (TAC) of ZAE and ZA-AgNPs. This method quantifies TAC by measuring the reduction of molybdenum (VI) to molybdenum (V), with maximal absorption at 695 nm.[60] The TAC values for AgNPs and ZAE were 45.38% and 38.59%, respectively, while ascorbic acid exhibited 94.13% antioxidant activity at the highest concentration of 200 µg/mL. The results are similar to those previously reported on *Ranunculus arvensis* L. extracts.[37] The results showed similar trends to the DPPH and RPA results: across the concentration range of 20–200 µg/mL, the antioxidant activity of the compounds increases accordingly (Fig.-5c). Chinnasamy et al. (2024) postulated that silver nanoparticles synthesised from aqueous extract of *Aristolochia bracteolata* also showed a concentration-dependent increase in the antioxidant activity.[58] AgNPs had an IC_{50} of 227.68 ± 0.002 µg/mL, less compared to ZAE (278.29 ± 0.001 µg/mL), although the standard had an IC_{50} of 81.56 ± 0.002 (Fig.-5d). AgNPs displayed higher TAC than the ZAE. Keshari *et al.* (2020) reported AgNPs synthesised from *Cestrum nocturnum* leaves exhibited better antioxidant activity than the sole extract.[14]

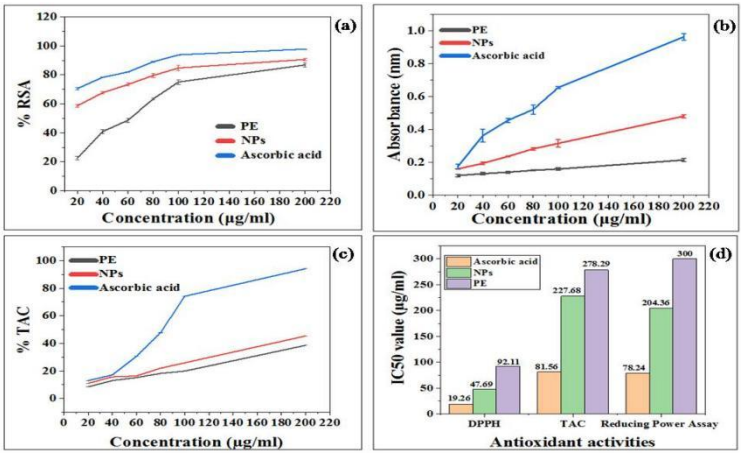

Fig.-4: Antioxidant Activity of Synthesised AgNPs, (a)DPPH, (b)RPA, (C) TAC, and (d) IC_{50} of DPPH, TAC, and Reducing Power Assay. Statistically Significant ($p < 0.01$). Values are Mean ± SD.

CONCLUSION

The present work describes an eco-friendly and cost-effective method for synthesising AgNPs using the leaf extract of *Z. armatum*, which demonstrated good antimicrobial and antioxidant properties compared to the crude extract. ZA-AgNPs were characterised using UV-Vis, DLS, FTIR, SEM, and XRD. This study highlights that ZA-AgNPs exhibit strong antimicrobial activity against gram-positive (*B. subtilis*) and gram-negative bacteria (*E. coli*) as well as the fungus *A. fumigatus*. Moreover, ZA-AgNPs showed significant reducing power and radical scavenging activity (DPPH), although the total antioxidant capacity (TAC) was less efficient. These findings suggest that *Z. armatum-mediated* AgNPs could be valuable in developing effective antimicrobial and antioxidant agents for modern medications. However, further in vitro and in vivo studies are needed to explore the natural potential of biosynthesised ZA-AgNPs.

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CONFLICTS OF INTEREST

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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REFERENCES

1. D. G. J. Larsson and C. F. Flach, *Nature Reviews Microbiology,* **20**, 257(2022), https://doi.org/10.1038/s41579-021-00649-x
2. T. Bekele and G. Alamnie, *Annals of Advances in Chemistry* **6**, 001(2022), https://doi.org/10.29328/journal.aac.1001025
3. A. Ghasempour, H. Dehghan, M. Ataee, B. Chen, Z. Zhao, M. Sedighi, X. Guo, and M. A. Shahbazi, *Molecules* **28**, 1(2023), https://doi.org/10.3390/molecules28093857
4. J. A. Dahl, B. L. S. Maddux, and J. E. Hutchison, *Chemical Reviews*, **107(6)**, 2228(2007), https://doi.org/10.1021/cr050943k
5. A. Haleem, M. Javaid, R. P. Singh, S. Rab, and R. Suman, *Global Health Journal,* **7**, 70(2023), https://doi.org/10.1016/j.glohj.2023.02.008
6. A. Bazrgaran, S. Mahmoodabadi, A. Ghasempour, E. Shafaie, A. Sahebkar, and S. Eghbali, *Plant Nano Biology,* **6**, 100052(2023), https://doi.org/10.1016/j.plana.2023.100052
7. M. Oves, M. Ahmar Rauf, M. Aslam, H. A. Qari, H. Sonbol, I. Ahmad, G. Sarwar Zaman, and M. Saeed, *Saudi Journal of Biological Sciences,* **29**, 460(2022), https://doi.org/10.1016/j.sjbs.2021.09.007
8. J. Flieger, W. Franus, R. Panek, M. Szymańska-Chargot, W. Flieger, M. Flieger, and P. Kołodziej, *Molecules,* **26**, 4986(2021), https://doi.org/10.3390/molecules26164986
9. D. Nath and P. Banerjee, *Environmental Toxicology and Pharmacology,* **36(3)**, 997(2013), https://doi.org/10.1016/j.etap.2013.09.002
10. P. Chakraborty, *Biochimie Open,***6***,* 9(2018), https://doi.org/10.1016/j.biopen.2017.12.003
11. A. U. Mirza, M. S. Khan, S. A. A. Nami, A. Kareem, S. Rehman, S. A. Bhat, and N. Nishat, *Chemistry and Biodiversity,* **16**, e1900145(2019), https://doi.org/10.1002/cbdv.201900145
12. N. S. Alharbi, N. S. Alsubhi, and A. I. Felimban, *Journal of Radiation Research and Applied Sciences,* **15**, 109(2022), https://doi.org/10.1016/j.jrras.2022.06.012
13. N. S. Kadam, D. B. Shelke, A. A. Naik, R. D. Tak, P. J. Doshi, and T. D. Nikam, *Plant Nano Biology,* **8**, 100071(2024), https://doi.org/10.1016/j.plana.2024.100071
14. A. K. Keshari, R. Srivastava, P. Singh, V. B. Yadav, and G. Nath, *Journal of Ayurveda and Integrative Medicine,* **11**, 37(2020), https://doi.org/10.1016/j.jaim.2017.11.003
15. I. Hussain, N. B. Singh, A. Singh, H. Singh, and S. C. Singh, *Biotechnology Letters,* **38***,* 545(2016), https://doi.org/10.1007/s10529-015-2026-7
16. C. P. Kala, N. A. Farooquee, and U. Dhar, *Conservation and Society,* **3**, 224(2005)
17. A. Bhatia, B. Singh, R. Arora, and S. Arora, BMC *Journal of Traditional and Complementary Medicine***19**, 74 (2019), https://doi.org/10.1186/s12906-019-2482-z
18. S. Jadoun, R. Arif, N. K. Jangid, and R. K. Meena, *Environmental Chemistry Letters*,**19**, 355–374(2021), https://doi.org/10.1007/s10311-020-01074-x
19. V. Bhatt, N. Kumar, U. Sharma, and B. Singh, *Separation Science Plus,* **1**, 311(2018),

https://doi.org/10.1002/sscp.201800004
20. N. Phuyal, P. K. Jha, P. Prasad Raturi, and S. Rajbhandary, *Journal of Ethnopharmacology*, **229**, 326(2019), https://doi.org/10.1016/j.jep.2018.08.010
21. R. Sahu, R. K. Kar, P. Sunita, P. Bose, P. Kumari, S. Bharti, S. Srivastava, and S. P. Pattanayak, *Journal of Ethnopharmacology,* **269**, 113758(2021), https://doi.org/10.1016/j.jep.2020.113758
22. M. Ibrar, N. Muhammad, A. Khan, P. J. Pharm Sci, S. Ali Khan, S. Zafar, S. Jan, N. Riaz, Z. Ullah, U. Farooq, and J. Hussain, *Pakistan Journal of Pharmaceutical Sciences,* **13,** 2017
23. M. Prasathkumar, S. Anisha, C. Dhrisya, R. Becky, and S. Sadhasivam, *Phytomedicine Plus,* 100029(2021), https://doi.org/10.1016/j.phyplu.2021.100029
24. K. K. Verma, B. Kumar, H. Raj, and A. Sharma, *Journal of Drug Delivery and Therapeutics,* **11**, 136(2021), https://doi.org/10.22270/jddt.v11i2-s.4786
25. N. Chelleng, M. Puzari, P. Chetia, and C. Tamuly, *Natural Product Research,* **37**, 1993(2023), https://doi.org/10.1080/14786419.2022.2110096
26. P. V. Patil, N. A. Nerlekar, A. R. Kuldeep, P. P. Patil, P. B. Dandge, T. D. Dongale, P. B. Dandge, and G. S. Rashinkar, *Plant Nano Bioogy,* **8**, 100069(2024), https://doi.org/10.1016/j.plana.2024.100069
27. M. Riaz, M. Altaf, P. Ahmad, M. U. Khandaker, H. Osman, E. M. Eed, and Y. Shakir, *Molecules,* **27**, 1166(2022), https://doi.org/10.3390/molecules27041166
28. M. Devi, S. Devi, V. Sharma, N. Rana, R. K. Bhatia, and A. K. Bhatt, *Journal of Traditional and Complementary Medicine*, **10**, 158(2020), https://doi.org/10.1016/j.jtcme.2019.04.007
29. M. Binsalah, S. Devanesan, M. S. Alsalhi, A. Nooh, O. Alghamdi, and N. Nooh*, Microorganisms,* **10**, 789(2022), https://doi.org/10.3390/microorganisms10040789
30. K. Jyoti and A. Singh, *Journal of Genetic Engineering and Biotechnology,* **14**, 311(2016), https://doi.org/10.1016/j.jgeb.2016.09.005
31. A. Umamaheswari, S. L. Prabu, S. A. John, and A. Puratchikody, *Biotechnology Reports,* **29**, e00595(2021), https://doi.org/10.1016/j.btre.2021.e00595
32. S. Ambika and M. Sundrarajan, *Journal of Photochemistry and Photobiology B: Biology,* **146**, 52(2015), https://doi.org/10.1016/j.jphotobiol.2015.02.020
33. E. . Armengol, M. Harmanci, and F. Laffleur, *Microbiological Research,* 126867(2021), https://doi.org/10.1016/j.micres.2021.126867
34. A. A. Mostafa, A. A. Al-Askar, K. S. Almaary, T. M. Dawoud, E. N. Sholkamy, and M. M. Bakri, *Saudi Journal of Biological Sciences,* **25**, 361(2018), https://doi.org/10.1016/j.sjbs.2017.02.004
35. A. Ahmad, S. Tandon, H. Tabassum, Z. Nooreen, I. Zareen Ahmad, U. Lindequist, R. A. Mothana, P. Alam, and N. Ali Siddiqui, *Journal of King Saud University-Science*, **34**, 101792(2022), https://doi.org/10.1016/j.jksus.2021.101792
36. Y. N. Mo, F. Cheng, Z. Yang, X. F. Shang, J. P. Liang, R. F. Shang, B. C. Hao, X. H. Wang, H. J. Zhang, A. Wali, C. F. Lu, and Y. Liu, *Frontiers of Veterinary Science,* **8**, 784898(2021), https://doi.org/10.3389/FVETS.2021.784898/BIBTEX
37. M. Z. Bhatti, A. Ali, A. Ahmad, A. Saeed, and S. A. Malik, *BMC Research Notes,* **8**, 279(2015), https://doi.org/10.1186/s13104-015-1228-3
38. M. Naseer, U. Aslam, B. Khalid, and B. Chen, *Scientific Reports,* **10**, 9055(2020), https://doi.org/10.1038/s41598-020-65949-3
39. M. Kumar, S. Jaast, and A. Kumar, *Asian Journal of Chemistry,* **37**, 2525(2025), https://doi.org/https://doi.org/10.14233/ajchem.2025.34478
40. S. Jaast and A. Grewal, *Current Research in Green and Sustainable Chemistry,* **4**, 100195(2021), https://doi.org/10.1016/j.crgsc.2021.100195
41. M. Zare-Bidaki, H. Aramjoo, Z. M. Mizwari, P. Mohammadparast-Tabas, R. Javanshir, and S. Mortazavi-Derazkola, *Arabian Journal of Chemistry,* **15**, 103842(2022), https://doi.org/10.1016/j.arabjc.2022.103842
42. D. Bharathi and V. Bhuvaneshwari, *Bionanoscience,* **9**, 155(2019), https://doi.org/10.1007/s12668-018-0577-5
43. R. Renuka, K. R. Devi, M. Sivakami, T. Thilagavathi, R. Uthrakumar, and K. Kaviyarasu,

Biocatalysis and Agricultural Biotechnology, **24**, 101567(2020), https://doi.org/10.1016/j.bcab.2020.101567
44. P. Sharma, R. Manchanda, R. Goswami, and S. Chawla, *Environmental Concerns and Sustainable Development: Volume 2: Biodiversity, Soil and Waste Management*, pp.27-44(2019), https://doi.org/10.1007/978-981-13-6358-0_2
45. V. Selvaraj, S. Sagadevan, L. Muthukrishnan, M. R. Johan, and J. Podder, *Journal of Nanostructure in Chemistry,* **9**, 153(2019), https://doi.org/10.1007/s40097-019-0306-9
46. R. Joshi, S. Aithal, A. More, V. Nema, and A. Mukherjee, *International Journal of Plant-Based Pharmaceuticals,* **4**, 101(2024), https://doi.org/10.62313/ijpbp.2024.217
47. T. C. Dakal, A. Kumar, R. S. Majumdar, and V. Yadav, *Frontiers in Microbiology,* **7**, 1(2016), https://doi.org/10.3389/fmicb.2016.01831
48. A. H. Hashem, E. Saied, B. H. Amin, F. O. Alotibi, A. A. Al-Askar, A. A. Arishi, F. M. Elkady, and M. A. Elbahnasawy, *Journal of Functional Biomaterials,* **13**, 242(2022), https://doi.org/10.3390/jfb13040242
49. N. Talank, H. Morad, H. Barabadi, F. Mojab, S. Amidi, F. Kobarfard, M. A. Mahjoub, K. Jounaki, N. Mohammadi, G. Salehi, M. Ashrafizadeh, and E. Mostafavi, *Talanta*, **243**, 123374(2022)
50. K. Chatterjee, J. Taneja, S. Khullar, and A. K. Pandey, *International Microbiology,* **26**, 143(2023), https://doi.org/10.1007/s10123-022-00280-7
51. S. Cintesun, M. M. Karpuz, M. F. Seyhan, and S. Marakli, *Chemistry Africa,* **8**, 3815(2025), https://doi.org/10.1007/s42250-025-01359-0
52. S. Sekhon-Loodu and H. P. V. Rupasinghe, *Frontiers in Nutrition,* **6**, 53(2019), https://doi.org/10.3389/fnut.2019.00053
53. M. M. Poojary, K. A. Vishnumurthy, and A. Vasudeva Adhikari, *Journal of Pharmaceutical Analysis,* **5**, 182(2015), https://doi.org/10.1016/j.jpha.2015.01.002
54. N. Kaur, R. Kumar, S. Alhan, H. Sharma, N. Singh, R. Yogi, V. Chhokar, V. Beniwal, M. Kumar Ghosh, S. Kumar Chandraker, S. Rustagi, and A. Kumar, *Inorganic Chemistry Communications,* **159**, 111735(2024), https://doi.org/10.1016/j.inoche.2023.111735
55. E. Sreelekha, B. George, A. Shyam, N. Sajina, and B. Mathew, *Bionanoscience,* **11**, 489(2021), https://doi.org/10.1007/s12668-021-00824-7
56. S. N. Kharat and V. D. Mendhulkar, *Materials Science and Engineering C,* **62**, 719(2016), https://doi.org/10.1016/j.msec.2016.02.024
57. P. Moteriya, H. Padalia, and S. Chanda, *Journal of Genetic Engineering and Biotechnology,* **15**, 505(2017), https://doi.org/10.1016/j.jgeb.2017.06.010
58. R. Chinnasamy, K. Chinnaperumal, T. Cherian, K. Thamilchelvan, B. Govindasamy, C. Vetrivel, V. Perumal, P. Willie, and P. Krutmuang, *Biomass Conversion and Biorefinery***, 14**, 28051(2024), https://doi.org/10.1007/s13399-023-03750-8
59. S. Salari, S. Esmaeilzadeh, and A. Samzadeh-kermani, *Iranian Journal of Pharmaceutical Research,* **18**, 430(2018)
60. M. Khatoon, E. Islam, R. Islam, A. A. Rahman, A. H. M. K. Alam, P. Khondkar, M. Rashid, and S. Parvin, *BMC Research Notes,* **6**, Article 121(2013), https://doi.org/10.1186/1756-0500-6-121

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