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Research Article

BIOMEDICAL APPLICATIONS OF GREEN SYNTHESIZED SILVER NANOPARTICLES FROM *PROSOPIS JULIFLORA*

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ABSTRACT

The present study explores the phytochemical properties and green synthesis of silver nanoparticles using the ethanol extract of *Prosopis juliflora*, a medicinal plant known for its bioactive constituents. Plant samples were aseptically collected from the Davangere district, Karnataka, and processed through washing, shade drying, and grinding. The crude extract was obtained using cold maceration with ethanol, followed by filtration and drying. Qualitative phytochemical screening confirmed the presence of secondary metabolites including phenols, tannins, saponins, and glycosides. UV-Visible spectrophotometry revealed an absorption peak at 434 nm, indicating the presence of bioactive compounds. FTIR spectroscopy identified functional groups involved in the biochemical profile of the extract. GC-MS analysis detected 35 secondary metabolites in the extract, with major constituents such as Ethane, 1,1-diethoxy-, Propane, 2,2-diethoxy-, and 4-O-Methylmannose. The green synthesis of silver nanoparticles was achieved by adding 1 mM $AgNO_3$ to the ethanol extract, confirmed by a color change from light brown to dark brown. The synthesized Pj-AgNPs were characterized by UV-Vis spectroscopy, showing a sharp peak at 401 nm. FTIR analysis of the nanoparticles revealed key functional groups involved in stabilization. SEM images confirmed spherical morphology, and EDAX analysis detected elemental silver along with carbon and oxygen. DLS analysis showed an average nanoparticle size of 70.21 nm. Antibacterial assays using the agar well diffusion method demonstrated significant inhibition, with the extract showing the highest activity against *Enterococcus faecalis* (25 mm) and Pj-AgNPs most effective against *Salmonella typhi* (19 mm). The findings suggest potential biomedical applications of Pj-AgNPs as natural antimicrobial agents.

Keywords: *Prosopis juliflora,* GC-MS, Pj-AgNPs, Antibacterial activity.

INTRODUCTION

Medicinal plants have been utilized since the beginning of human civilization to treat various diseases. Each plant exhibits unique therapeutic properties, largely due to the increasing interest of researchers in exploring natural resources for improving human health. Among these, species belonging to the genus *Prosopis* have been widely used in traditional and folk medicine. The genus *Prosopis*, belonging to the Fabaceae (or Leguminosae) family, comprises approximately 45 species of spiny trees and shrubs. These species are native to both subtropical and tropical regions around the world. Traditionally, *Prosopis* species have been employed to treat a broad spectrum of ailments including asthma, postpartum pain, callouses, conjunctivitis, diabetes, diarrhea, fever, flu, liver infections, malaria, otitis, rheumatism, scabies, skin inflammation, spasms, stomach ache, and the removal of bladder and pancreatic stones. Furthermore, they have been used as expectorants, painkillers, and treatments for birth complications and lactation issues.

Beyond their traditional applications, *Prosopis* species are of significant commercial interest. Various parts of these plants including their paste, gum, leaves, and pods have demonstrated diverse bioactive properties, such as

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anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antioxidant activities. These therapeutic effects are attributed to their rich phytochemical content, including flavonoids, tannins, alkaloids, quinones, and phenolic compounds. Notably, *Prosopis* plants are known for their high levels of phenolic compounds, including anthocyanins and flavonoids such as apigenin, luteolin, and quercetin, along with their derivatives (Sharifi-Rad *et al*., 2019; Henciya *et al*., 2016). In developing countries, research into herbal medicine has surged, as it helps preserve traditional knowledge while providing affordable healthcare alternatives. As traditional medicine gains global recognition, screening medicinal plants for bioactive compounds has become increasingly important. To date, over 12,000 bioactive compounds have been isolated from plants representing less than 10% of the total estimated number (Cowan, 1999). According to World Health Organization (WHO) statistics from 2000, over 60% of the world's population relies on traditional medicine for their primary healthcare needs.

In the United Arab Emirates (UAE), *P. juliflora*, locally known as "Al Ghwaif," is a particularly notable species. *P. juliflora*, a member of the Leguminosae family, is also commonly found in arid and semi-arid regions of India and has been widely used in traditional medicine. It has been employed for treating conditions such as catarrh, colds, diarrhea, dysentery, flu, inflammation, measles, sore throat, and wound healing (Hartwell, 1971; Saadoun *et al*., 2014). *P. juliflora* is a fast-growing, thorny, deciduous, and drought-resistant plant characterized by a wide crown and deep root system. Though originally native to Central and South America including southern Mexico, Panama, the Caribbean, and northern South America it has become an invasive species in India, where it competes with native vegetation. It thrives in a wide range of soil types, including degraded and wasteland soils, and grows at altitudes ranging from sea level to 1,500 meters. Due to its extensive ethnobotanical applications, *P. juliflora* holds a significant position in traditional medicine. It has been used to treat ailments such as toothache, asthma, bronchitis, conjunctivitis, skin disorders, blood and venereal diseases, and is also known for its insecticidal properties (Thakur *et al*., 2015).

The plant's various parts leaves, seeds, bark, and flowers have been traditionally used for the treatment of a wide range of conditions, including hepatic and ophthalmic disorders. Decoctions prepared from the leaves and seeds have been reported to be effective in wound healing and treating gastrointestinal issues. Several pharmacologically significant alkaloids have been identified in this plant, including 3'-oxojuliprosine, s*ceojuliprosopinol*, *juliflorine*, *julifloricine*, *julifloridine*, *juliprosine*, *juliprosinene*, and *juliflorinine* (Singh *et al*., 2014). Epidemiological studies have shown a negative correlation between the incidence of chronic diseases and the intake of phytochemicals, such as carotenoids and phenolics. *Prosopis* species are particularly rich in these compounds, which include alkaloids, phenolic acids, flavonoids, glycosides, steroids, tannins, and triterpenoids all recognized for their health-promoting effects. Many research efforts have focused on the isolation, identification, and quantification of these bioactive constituents from various *Prosopis* species.

Ethnopharmacological surveys have identified species such as *P. africana*, *P. alba*, *P. cineraria*, *P. farcta*, *P. glandulosa*, *P. juliflora*, *P. nigra*, *P. ruscifolia*, and *P. spicigera* as some of the most widely used *Prosopis* species in traditional medicine worldwide. Among these, *P. cineraria*, *P. juliflora*, and *P. africana* have received special attention due to their well-documented medicinal properties. *P. juliflora* is extensively used both orally and topically. The leaves, gum, flowers, bark, and whole plant are employed in treating various conditions such as boils, eye inflammation, muscular pain, kidney stones, toothache, asthma, cough, and even breast cancer. It is frequently used in several regions of India, including the Thar Desert (Sindh), Bahawalnagar (Punjab), and Mohmand Agency (FATA), as well as in western Madhya Pradesh where its stem bark is used in asthma treatment (Sharifi-Rad *et al*., 2019; Henciya *et al*., 2016).

Beyond medicinal uses, *P. juliflora* also serves practical and nutritional purposes. It is used as livestock fodder, construction wood, and its pods are consumed as food or processed into coffee substitutes, syrup, and fermented beverages due to their high sugar content. Scientific reports have highlighted its antioxidant, antiparasitic, antimicrobial, and antitumor potentials. It has also been observed that plants thriving under harsh environmental conditions tend to produce greater quantities of secondary metabolites compounds that can be leveraged for therapeutic applications. Despite its many uses, the anticancer properties of *P. juliflora*, particularly from its leaf extracts, remain underexplored. Therefore, a recent study investigated the antioxidant and anticancer potential of *Prosopis juliflora* methanolic leaf extract (PJME) against prostate cancer cell lines (Khan *et al*., 2022). Moreover, the ethanol extract of *P. juliflora* has been employed in the green synthesis and characterization of silver nanoparticles (AgNPs) to explore their biomedical applications.

Nanotechnology has emerged as one of the fastest-growing scientific fields due to its wide-ranging applications across various industries. It focuses on the synthesis and manipulation of nanoparticles (NPs), which can be generated through natural or artificial methods (Rahi *et al*., 2014). Traditional chemical and physical methods of NP synthesis are often inefficient, environmentally harmful, and expensive (Barkhade, 2018). There are two primary approaches for NP synthesis: the bottom-up and top-down methods. Bottom-up techniques include solvothermal synthesis, co-precipitation, sonochemistry, pyrolysis, and green synthesis, while top-down approaches involve techniques such as laser ablation, spray pyrolysis, and ball milling. However, many of these conventional methods are time-consuming and environmentally damaging.

To address these challenges, researchers have increasingly adopted biological synthesis methods using

microorganisms and plants. Among these, plant-mediated green synthesis has gained significant momentum for being eco-friendly and cost-effective. Recent developments in this field have led to the sustainable fabrication of nanoscale materials with enhanced biological properties (Al-Radadi *et al*., 2022; Khan *et al*., 2022; Zafar *et al*., 2022; Jan *et al*., 2020). Nanoparticles are defined as materials with at least one dimension between 1 and 100 nanometers. Metal and metal oxide nanoparticles such as silver (Ag), copper (Cu), gold (Au), zinc oxide (ZnO), selenium (Se), and copper oxide (CuO) have shown great promise in various applications, particularly in overcoming antimicrobial resistance and improving targeted drug delivery in cancer therapy. The size, shape, and surface area of these nanoparticles significantly influence their bioactivity (Jan *et al*., 2021). Green-synthesized nanoparticles offer a new platform for developing safer, targeted therapeutic agents. In particular, silver nanoparticles produced using plant extracts have demonstrated potent antibacterial, anti-inflammatory, wound-healing, anticancer, and bioimaging capabilities (Faisal *et al*., 2022; Jan *et al*., 2021). These advancements position green nanotechnology as a pivotal approach for future medical and industrial innovations.

In this research, the phytochemical properties and green synthesis of silver nanoparticles using ethanol extract of *Prosopis juliflora*, a medicinal plant rich in bioactive compounds, were investigated. The crude extract was prepared through cold maceration with ethanol, followed by filtration and drying. Qualitative phytochemical screening revealed the presence of secondary metabolites such as phenols, tannins, saponins, and glycosides. The extract was analyzed using UV-Visible spectrophotometry, FTIR, and GC-MS. AgNPs were synthesized by adding 1 mM $AgNO_3$, indicated by a color change. The Pj-AgNPs were characterized using UV-Vis, FTIR, SEM, EDAX, and DLS, and antibacterial activity was assessed via agar well diffusion.

MATERIALS AND METHODS

Collection of plant sample

Healthy sample of *Prosopis juliflora* were aseptically collected, photographed for documentation, and stored in paper bags for further analysis (Kumar *et al*., 2017).

Preparation of plant sample

Prosopis juliflora plant samples were carefully washed, shade-dried, and ground into a fine powder using a mechanical grinder. The resulting powdered material was stored in zip-lock polythene bags for subsequent extraction processes. (Kumar *et al*., 2017).

Extraction of Phytochemicals from *Prosopis juliflora*

The crude compound from *Prosopis juliflora* was extracted using the cold maceration method. The pulverized plant material was immersed in ethanol and agitated for 48–72 hours. The solvent containing the phyto-compounds was then separated and filtered. After evaporation of the solvent, the crude extract was dried and collected. The extract was further analyzed using UV-Visible spectrophotometry and FTIR spectroscopy. GC-MS analysis. (Garg and Garg, 2018. Dadayya *et al*., 2023b,)

Qualitative Screening of Secondary Metabolites in *Prosopis juliflora* Ethanol Extract

The presence of various secondary metabolites in the ethanol extract of *Prosopis juliflora* was identified using standard phytochemical screening protocols as described by Gautam *et al*. (2020); Subhakar *et al*., (2025).

Test for Phenols (Ferric Chloride Test)

To 2 mL of plant extract, 5% ferric chloride solution was added. The development of a deep blue or black coloration indicated the presence of phenolic compounds.

Test for Tannins (Gelatin Test)

A mixture of plant extract, gelatin, and 10% sodium chloride was prepared. The formation of a white precipitate confirmed the presence of tannins.

Test for Alkaloids (Mayer's Test)

To 2 mL of the extract, 2 mL of Mayer's reagent was added. The appearance of a dull white or cream-colored precipitate indicated the presence of alkaloids.

Test for Flavonoids (Alkaline Reagent Test)

About 2 mL of plant extract was treated with 20% sodium hydroxide solution. The development of an intense yellow coloration confirmed the presence of flavonoids.

Test for Terpenoids (Salkowski's Test)

To 2 mL of extract, 2 mL of chloroform and 2 mL of concentrated sulfuric acid were added. A reddish-brown coloration at the interface confirmed the presence of terpenoids.

Test for Steroids (Liebermann-Burchard Test)

To 0.5 g of plant extract, 2 mL of acetic anhydride and 2 mL of concentrated sulfuric acid were added. A color change from violet to blue or green indicated the presence of steroids.

Test for Saponins (Foam Test)

The plant extract was vigorously shaken with 20 mL of distilled water in a graduated cylinder for 15 minutes. The formation of a persistent 1 cm foam layer indicated the presence of saponins.

Test for Glycosides (Keller-Killiani Test)

To 2 mL of extract, glacial acetic acid containing ferric chloride was added, followed by concentrated sulfuric acid.

The formation of a brown ring at the interface confirmed the presence of glycosides.

Test for Proteins and Amino Acids (Ninhydrin Test)

To 2 mL of plant extract, 3–5 drops of freshly prepared 2% ninhydrin reagent were added, and the mixture was heated in a water bath. The appearance of a blue coloration indicated the presence of proteins and amino acids.

Test for Carbohydrates (Fehling's Test)

Equal volumes of Fehling's solution A and B were mixed with 2 mL of plant extract and heated in a boiling water bath. The formation of a brick-red precipitate confirmed the presence of carbohydrates.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of *Prosopis juliflora* Ethanol Extract

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify and characterize the secondary metabolites present in the ethanol extract of *Prosopis juliflora*. The analysis was carried out using a Rtx®-5 capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Prior to injection, the sample was filtered through a Whatman No.1 filter paper (0.2 µm pore size). High-purity helium gas (99.999%) was used as the carrier at a flow rate of 1 mL/min in split mode. A 1 µL aliquot of the extract was injected at an inlet temperature of 280°C. The oven temperature was programmed to start at 70°C (held for 2 minutes) and then ramped at a rate of 7°C/min up to a final temperature of 320°C. The ion source temperature was set at 250°C, and mass spectra were acquired using electron ionization (EI) at 70 eV. The detector operated in scan mode over a mass range of 30–500 Da. The total run time was 22.5 minutes, including a 3-minute solvent delay. Compound identification was achieved by comparing the obtained mass spectra with entries in the NIST23-l library database (Kanjana *et al*., 2019; Konappa *et al*., 2020).

Biogenic Synthesis and Characterization of *Prosopis juliflora*-Synthesized Silver Nanoparticles (Pj-AgNPs)

The biogenic synthesis of silver nanoparticles (Pj-AgNPs) was carried out using *Prosopis juliflora* ethanol extract. A 1 mM silver nitrate solution was mixed in a 1:1 ratio with the plant extract and incubated in the dark at 28 ± 2 °C for 24 hours. The formation of nanoparticles was visually confirmed by a noticeable color change and further validated using UV-Visible spectroscopy within the 200–800 nm wavelength range. The synthesized nanoparticles were purified by centrifugation at 15,000 rpm for 15 minutes, followed by repeated washing with sterile distilled water. The resulting pellet was then dried at 50 °C for 24 hours. Characterization of PJ-AgNPs was performed using Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups, Scanning Electron Microscopy with Energy-Dispersive X-ray Analysis (SEM-EDAX) for surface morphology and elemental composition, and Dynamic Light Scattering (DLS) to assess particle size distribution. (Hemlata *et al*., 2020; Khatun *et al*., 2023; Asif *et al*., 2022; Lima *et al*., 2024).

Antibacterial Activity of *Prosopis juliflora* Extract and Pj-AgNPs Using Agar Well Diffusion Method

The antibacterial potential of *Prosopis juliflora* extract and Pj-AgNPs was evaluated using the agar well diffusion method against three pathogenic bacterial strains: *Escherichia coli* (MTCC 1559), *Enterococcus faecalis* (MTCC 439), and *Salmonella typhi* (MTCC 734). Test bacteria were aseptically inoculated into sterile Mueller–Hinton broth and incubated at 37°C for 18 hours. The resulting cultures were swabbed onto sterile Mueller–Hinton agar plates, and wells were created using a sterile cork borer. The standard antibiotic, chloramphenicol, Streptomycin, and Ciprofloxacin (1 mg/mL in sterile distilled water), served as a positive control, while DMSO was used as a negative control. Test samples were dissolved in DMSO at concentrations of 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL. A volume of 100 µL of each test solution and control was pipetted into the wells. Plates were left undisturbed for 30 minutes to allow diffusion, followed by incubation at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the zone of inhibition around each well using a zone scale. The size of the inhibition zone directly reflected the efficacy of the test samples against the pathogenic bacteria. (Manandhar *et al*., 2019; Dadayya *et al*., 2023b, Dadayya *et al*., 2023c, Dadayya *et al*., 2025).

RESULTS AND DISCUSSION

Prosopis juliflora plant samples were aseptically collected from Davangere district, Karnataka (Figure1). The samples were photographed (Figure 2a and b), thoroughly washed, shade-dried, and ground into a fine powder using a mechanical grinder. The powdered material was then stored in zip-lock bags for further extraction procedures (Figure 2c). The crude extract of *Prosopis juliflora* was prepared through cold maceration using ethanol, followed by filtration and drying. The extract was then subjected to qualitative screening for secondary metabolites and further analyzed using UV-Visible spectrophotometry and FTIR to identify the presence of phytochemicals. This work investigates the phytochemical characteristics of medicinal plants, encompassing bioactive components such as proteins, amino acids, alkaloids, flavonoids, terpenoids, phenols, and tannins. Qualitative phytochemical screening was used to confirm the presence of phenols, tannins, saponins and glycosides in the ethanolic extract of *Prosopis juliflora* (Table 1). UV-Visible spectrophotometry is widely used for the preliminary identification of bioactive secondary metabolites. In this study, it was utilized to examine the secondary metabolites in the ethanol extract of *Prosopis juliflora*, revealing an absorption peak at 434 nm within the 200–800 nm wavelength range (Figure. 3).

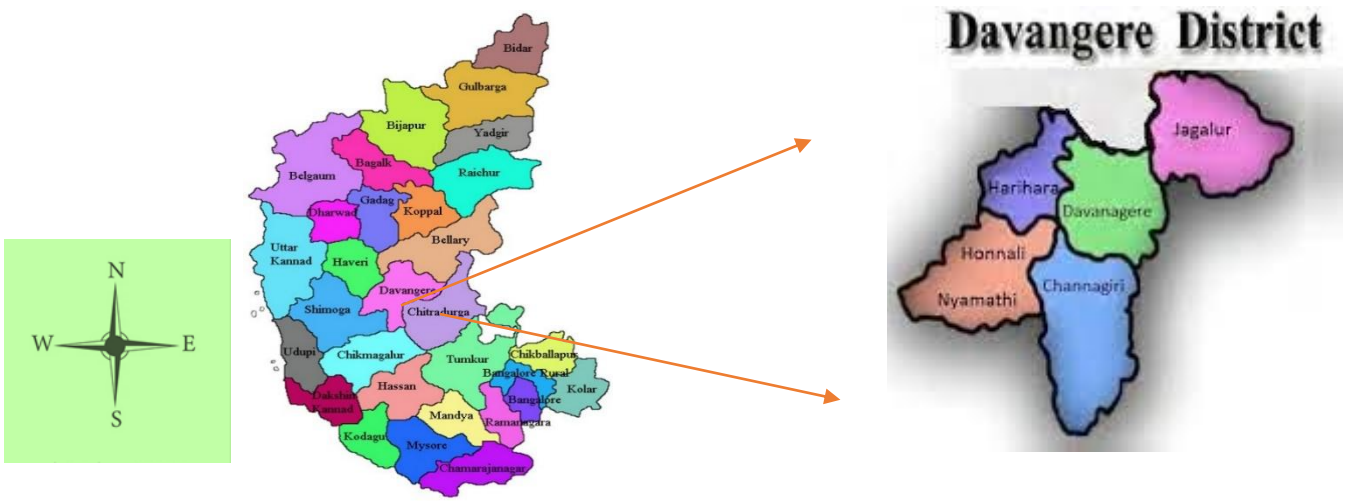

Figure. 1. Collection of plant samples in Davagere district, Karnataka.

Figure 2. (a) *Prosopis juliflora* plant sample; (b) Powdered form of *Prosopis juliflora* used for extraction. (c) Sample stored on zip-lock bags.

Table. 1. Phytochemicals in *Prosopis juliflora* extract.

Sl. No	Phytochemicals	Plant sample
1	Phenols	Present
2	Tannins	Absent
3	Alkaloids	Present
4	Flavonoids	Present
5	Terpenoids	Absent
6	Steroids	Absent
7	Saponins	Present
8.	Glycosides	Absent
9	Proteins/Amino acids	Absent
10	Carbohydrates	Absent

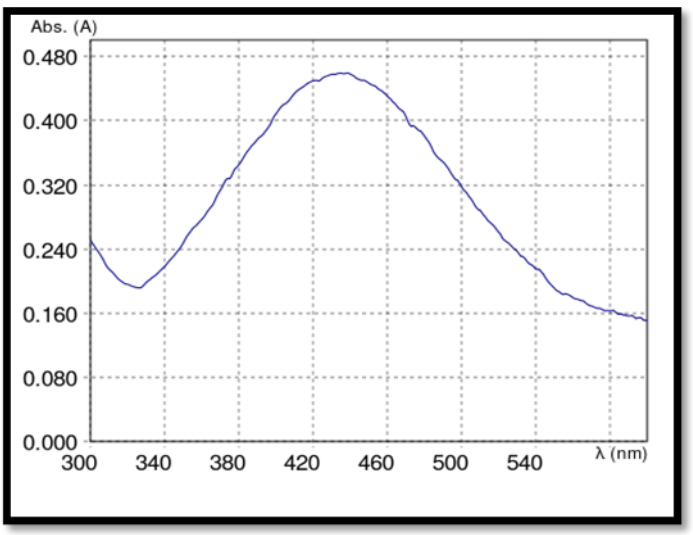

Figure. 3. UV-Visible spectrophotometry of *Prosopis juliflora* ethanol extract.

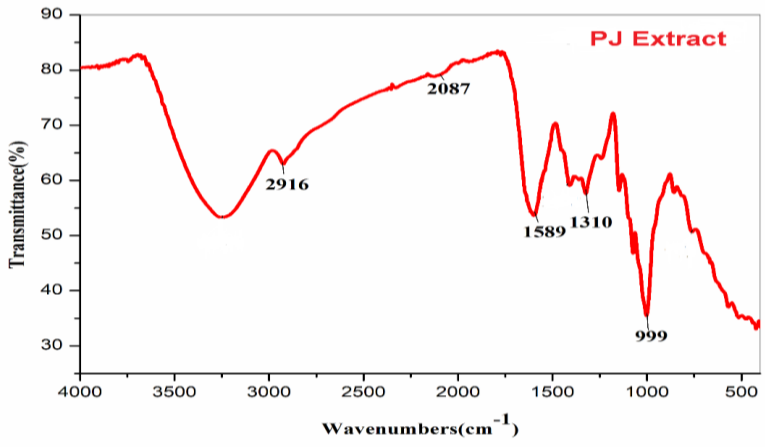

Figure. 4. FTIR pattern of *Prosopis juliflora* ethanol extract.

Table 2. Functional groups of *Prosopis juliflora* ethanol extract

Functional groups	Wavelength detected(cm^{-1})
Methyl C-H asym.\ sym. stretch	2916
Aromatic combination bands	2087
C=C-C Aromatic ring stretch	1589
Phenol or tertiary alcohol. OH bend	1310
Silicate ion	999

Fourier Transform Infrared (FTIR) spectroscopy was employed to determine the functional groups present in the ethanol extract of *Prosopis juliflora*, with the results illustrated in the accompanying figure 4 and table 2. Gas chromatography–mass spectrometry (GC-MS) analysis of the ethanol extract of *Prosopis juliflora* identified 35 bioactive secondary metabolites by comparison with the NIST23s-lib database (Table 3 and Figure. 5). The major compounds detected were Ethane, 1,1-diethoxy- at peak 1 (RT 2.836, area percentage 39.14%), Propane, 2,2-diethoxy- at peak 4 (RT 3.309, area percentage 41.10%), and 4-O-Methylmannose at peak 23 (RT 16.257, area percentage 5.08%). These bioactive compounds exhibit various biological activities and contribute to the synthesis of silver nanoparticles.

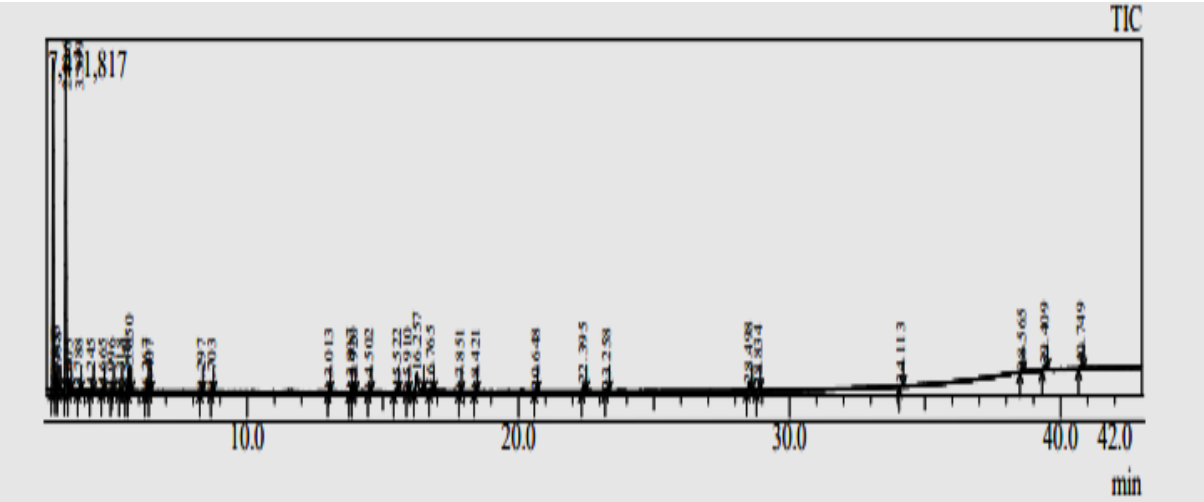

Figure 5. GC-MS Chromatogram ethanol extract of *Prosopis juliflora.*

Table 3. List of identified bioactive compounds ethanol extract of *Prosopis juliflora.*

Sl. No	Compound	Molecular formula	Molecular weight	Retention n time	Peak	Area (%)
01.	Ethane,1,1-diethoxy-	$C_6H_{14}O_2$	118.17 g/mol	2.836	1	39.14
02.	1-Butanol,3-methyl-	$C_5H_{12}O$	88.15 g/mol	2.953	2	1.84
03.	1-Butanol, 3-methyl	$C_5H_{12}O$	88.15 g/mol	2.998	3	0.71
04.	Propane, 2,2-diethoxy-	$C_7H_{16}O_2$	132.20 g/mol	3.309	4	41.10
05.	Isobutyl acetate	$C_6H_{12}O_2$	116.19 g/mol	3.405	5	0.17
06.	1-Propene, 3,3-diethoxy-	$C_7H_{14}O_2$	130.18 g/mol	3.788	6	0.30
07.	Propane, 2-ethoxy-	$C_5H_{10}O$	86.13 g/mol	4.245	7	0.28
08.	Propane, 1,1-diethoxy-2- methyl-	$C_8H_{18}O_2$	146.22 g/mol	4.665	8	0.30
09.	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130.18 g/mol	4.996	9	0.13
10.	Propane, 1-(1-methylethoxy)-	$C_6H_{14}O$	102.17 g/mol	5.318	10	0.24

11.	Ethyl 3-ethoxyacrylate	$C_7H_{12}O_3$	144.17 g/mol	5.549	11	0.27
12.	Ethyl 3-ethoxyacrylate	$C_7H_{12}O$	144.17 g/mol	5.650	12	2.71
13.	Butane, 1,1 diethoxy-3-methyl-	$C_9H_{20}O_2$	160.25 g/mol	6.267	13	0.28
14.	3,3-Diethoxybutan-2-one	$C_8H_{16}O_3$	160.21 g/mol	6.407	14	0.16
15.	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176.25 g/mol	8.297	15	0.14
16	(Methoxymethyl)trimethylsila ne	$C_5H_{14}OSi$	118.25 g/mol	8.703	16	0.14
17	1-Tetradecanol	$C_{14}H_{30}O$	214.387 g/mol	13.013	17	0.12
18	Cyclohexane, octyl-	$C_{14}H_{28}$	196.37 g/mol	13.807	18	0.13
19	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	519.078 g/mol	13.920	19	0.23
20	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32 g/mol	14.502	20	0.22
21	n-Pentadecanol	$C_{15}H_{32}O$	228.42 g/mol	15.522	21	0.54
22	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.23 g/mol	15.910	22	0.11
23	4-O-Methylmannose	$C_7H_{14}O_6$	194.18 g/mol	16.257	23	5.08
24	4-O-Methylmannose	$C_7H_{14}O_6$	194.18 g/mol	16.765	24	0.66
25	Behenic alcohol	$C_{22}H_{46}O$	326.61 g/mol	17.851	25	0.13
26	Neophytadiene	$C_{20}H_{38}$	278.52 g/mol	18.421	26	0.21
27	n-Nonadecanol-1	$C_{19}H_{40}O$	284.51 g/mol	20.648	27	0.16
28	Phytol	$C_{20}H_{40}O$	296.53 g/mol	22.395	28	0.81
29	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	$C_{20}H_{34}O_2$	306.47 g/mol	23.258	29	0.19
30	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoa	$C_{28}H_{34}N_2O_3$	446.57 g/mol	28.498	30	0.26
31	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561g/mol	28.834	31	0.34
32	Tetrapentacontane	$C_{54}H_{11}O$	759.4512g/mol	34.113	32	0.20
33	Stigmasterol	$C_{29}H_{48}O$	412.69 g/mol	38.565	33	0.26
34	Gamma. -Sitosterol	$C_{29}H_{50}O$	414.71 g/mol	39.409	34	1.02
35	Lupeol	$C_{30}H_{50}O$	426.7174g/mol	40.749	35	0.45

The formation of Pj-AgNPs was visually confirmed by a colour change in the reaction mixture from light brown to dark brown after the addition of 1 mM $AgNO_3$ to the *Prosopis juliflora* ethanol extract (Figure. 6a), indicating the reduction of Ag^+ to Ag^0 at room temperature. The synthesized nanoparticles were purified by centrifugation at 15,000 rpm for 15 minutes and washed repeatedly with sterile distilled water. The resulting pellet was dried at 50 °C for 24 hours (Figure 6b). The PJ-AgNPs were subsequently characterized using FTIR to identify functional groups, SEM-EDAX for morphological and elemental analysis, and DLS for particle size determination.

Figure. 6. (a) Colour change from light brown to dark brown after treatment with 1 mM $AgNO_3$, indicating the formation of Pj-AgNPs. (b) Crystalline form of synthesized Pj-AgNPs.

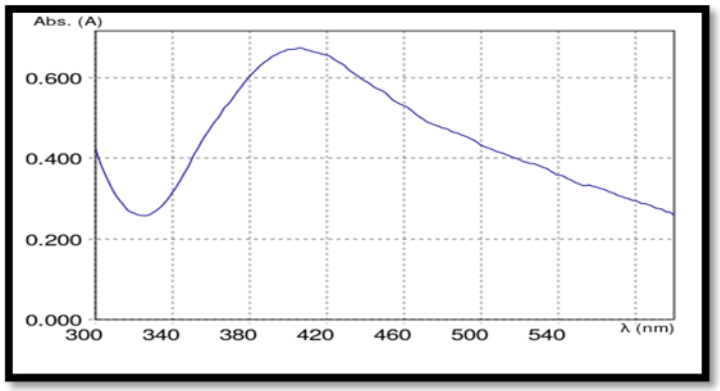

Figure 7. UV-Visible spectrometer of Pj-AgNPs

The absorbance of the sample supernatant was measured using a UV-Visible spectrophotometer. The absorption spectrum displayed a distinct peak at 401 nm, confirming the successful synthesis of Pj-AgNPs, as shown in the figure 7. FTIR analysis was performed to examine the interaction between silver ions and the bioactive compounds in the *Prosopis juliflora* ethanol extract responsible for stabilizing Pj-AgNPs. The FTIR spectra of the synthesized nanoparticles revealed peaks corresponding to different functional groups, as illustrated in the accompanying table and figure 8.

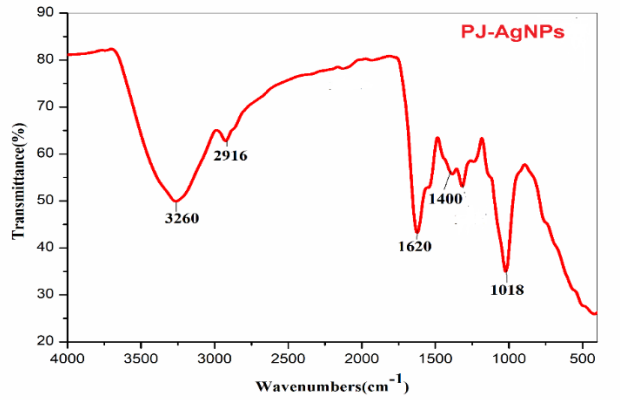

Figure 8. FTIR pattern of biosynthesized Pj-AgNPs.

Table 4. Functional groups of Pj-AgNPs.

Functional groups	Wavelength detected(cm^{-1})
Hydroxy group, H-bonded OH stretch	3260
Methyl C-H asym.\ sym. stretch	2916
Primary amine, NH bend	1620
Phenol, or Tertiary alcohol, OH bend	1400
Primary amine, CH stretch	1018

The SEM analysis showed that the synthesized Pj-AgNPs exhibited a predominantly spherical morphology, as depicted in the corresponding figure 9.

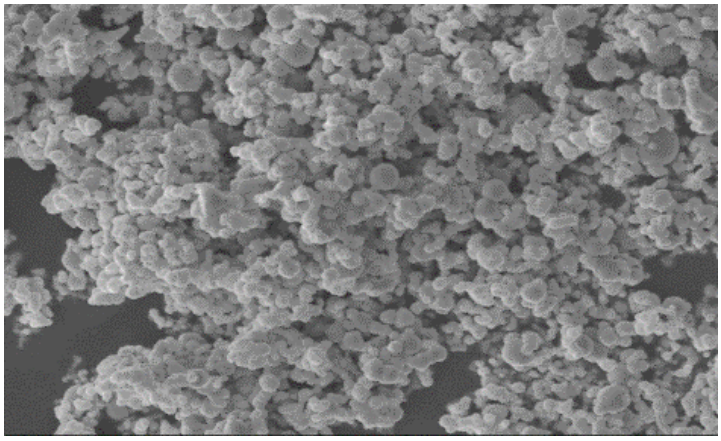

Figure 9. SEM image of biosynthesized Pj-AgNPs.

The EDAX spectrum of the synthesized nanoparticles displayed a strong signal for silver atoms around 3 keV, confirming the successful reduction of silver ions to elemental silver in Pj-AgNPs, along with weaker signals for carbon and oxygen atoms (Figure 10).

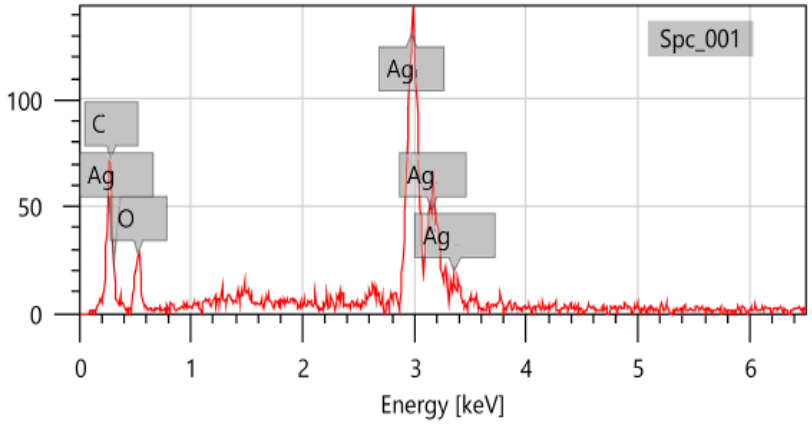

Figure. 10. EDAX pattern of biosynthesized Pj-AgNPs.

Dynamic Light Scattering (DLS) analysis showed that the synthesized Pj-AgNPs had an average particle size of 70.21 nm. The appearance of a single, sharp peak indicated the high purity of the nanoparticles (Figure. 11).

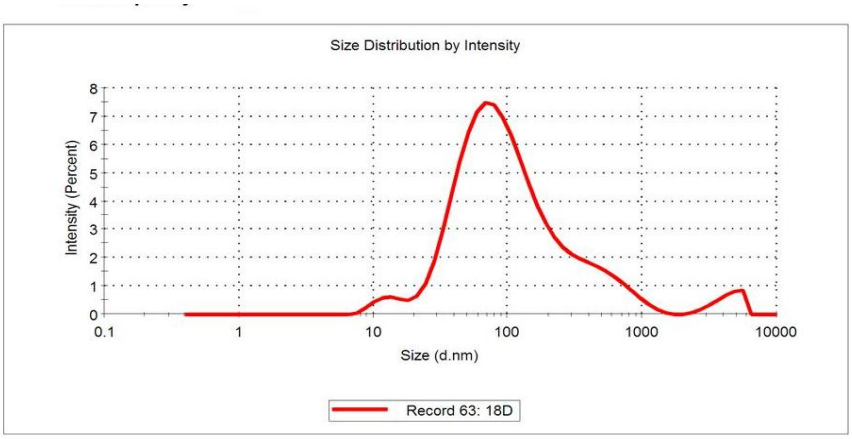

Figure 11. DLS pattern of biosynthesized Pj-AgNPs.

Using the agar well diffusion method, the antibacterial activity of *Prosopis juliflora* extract and Pj-AgNPs was evaluated against *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella typhi*. The *Prosopis juliflora* extract exhibited the highest zone of inhibition against *Enterococcus faecalis* (25 mm), while Pj-AgNPs showed the maximum inhibitory effect against *Salmonella typhi* (19 mm). The antibacterial activity against the remaining organisms is summarized in the accompanying table 5,6 and figure 12, 13 and 14.

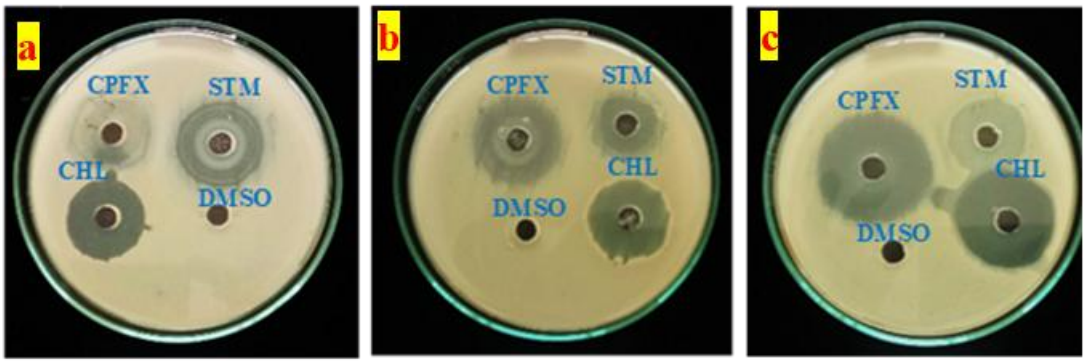

Figure 12. Standard antibiotics of antibacterial activity against test bacteria pathogens (a) *Salmonella typhi* (b) *Escherichia coli,* (c *Enterococcus faecalis.*

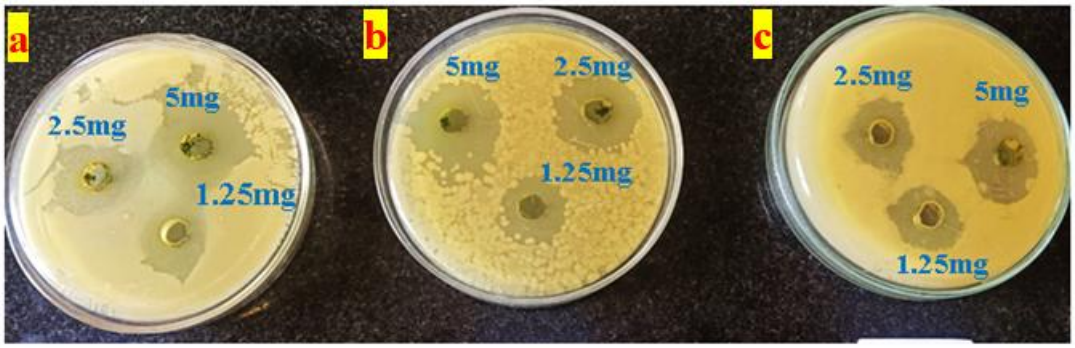

Figure 13. Antibacterial activity of *Prosopis juliflora* extract against bacterial pathogens (a) *Salmonella typhi* (b) *Escherichia coli,* (c) *Enterococcus faecalis.*

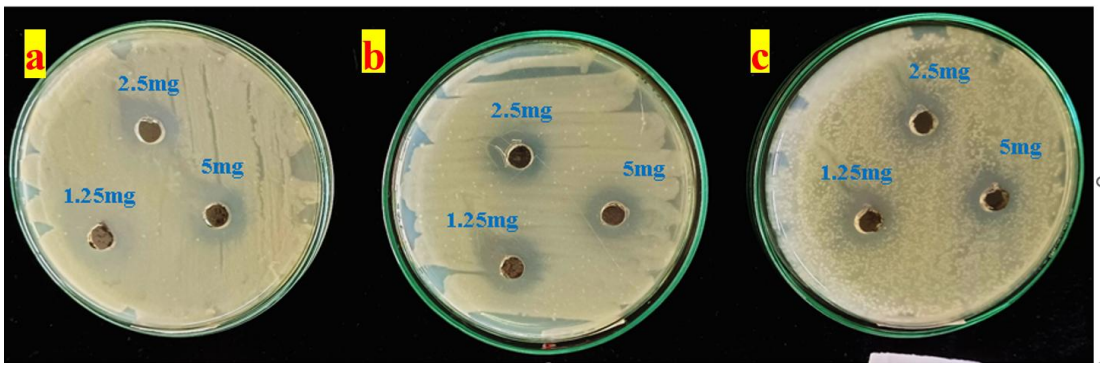

Figure 14. Antibacterial activity of *Pj-AgNPs* against bacterial pathogens (a) *Salmonella typhi* (b) *Escherichia coli,* (c) *Enterococcus faecalis.*

Table 5. Zone of inhibition values of antibacterial activity of *Prosopis juliflora* extract at different concentrations against bacterial pathogens.

Test pathogenic Bacteria	Measurement of zone of inhibition in diameter (mm)					
	Standard Antibiotics			*Prosopis juliflora* extract(mg/ml)		
	Streptomycin (STM)	Ciprofloxacin (CPFX)	Chloramphenicol (CHL)	5mg/ml	2.5mg/ml	1.25mg/ml
Salmonella typhi	23. mm	25 mm	25 mm	21 mm	19 mm	18 mm
Escherichia coli	21. mm	27 mm	25 mm	23 mm	22 mm	18 mm
Enterococcus faecalis	23. mm	35 mm	29 mm	25 mm	17 mm	15 mm

Table 6. Zone of inhibition values of antibacterial activity of *Pj-AgNPs* at different concentrations against bacterial pathogens.

Test pathogenic Bacteria	Measurement of zone of inhibition in diameter (mm)					
	Standard Antibiotics			*Pj-AgNPs* (mg/ml)		
	Streptomycin (STM)	Ciprofloxacin (CPFX)	Chloramphenicol (CHL)	5mg/ml	2.5mg/ml	1.25mg/ml
Salmonella typhi	23. mm	25 mm	25 mm	19 mm	17 mm	13 mm
Escherichia coli	21. mm	27 mm	25 mm	16 mm	14 mm	12 mm
Enterococcus faecalis	23. mm	35 mm	29 mm	17 mm	15 mm	13 mm

The present study emphasizes the phytochemical richness and potential biomedical applications of *Prosopis juliflora*, particularly in the green synthesis of silver nanoparticles (PJ-AgNPs). The traditional use of *Prosopis* species in folk medicine for treating a wide range of ailments underlines their therapeutic value. Consistent with this ethnobotanical significance, the phytochemical analysis of the ethanol extract of *P. juliflora* confirmed the presence of secondary metabolites such as phenols, tannins, saponins, and glycosides compounds well known for their biological activity. UV-Visible spectrophotometry revealed a notable absorption peak at 434 nm, indicating the presence of bioactive compounds capable of participating in nanoparticle synthesis. FTIR analysis provided further evidence by identifying functional groups involved in the stabilization of silver nanoparticles. GC-MS profiling revealed 35 bioactive compounds, with Ethane,1,1-diethoxy- and Propane,2,2-diethoxy- being the dominant constituents. These compounds likely played a key role in reducing Ag^{+} ions to elemental silver (Ag^{0}) and stabilizing the resulting nanoparticles.

The formation of PJ-AgNPs was visually confirmed by a distinct color change and further substantiated by an absorption peak at 401 nm in the UV-Vis spectrum. FTIR analysis of PJ-AgNPs identified key functional groups that contributed to the stabilization of the nanoparticles. SEM imaging showed that the synthesized nanoparticles were predominantly spherical in shape, and EDAX confirmed the presence of elemental silver through a strong signal around 3 keV. DLS analysis indicated an average particle size of 70.21 nm and the presence of a single peak, suggesting high nanoparticle purity and stability. The antimicrobial efficacy of both the *P. juliflora* extract and PJ-AgNPs was demonstrated against common bacterial pathogens. The extract showed the strongest inhibition against *Enterococcus faecalis*, while PJ-AgNPs exhibited enhanced activity against *Salmonella typhi*. These findings underscore the synergistic effect of combining plant-based phytochemicals with nanotechnology to develop effective antimicrobial agents.

Comparative analysis with other studies further supports the findings. For instance, *Prosopis juliflora* extracts have previously shown antibacterial and cytotoxic activities, as noted by Asaad Khalid *et al*. (2024). Similarly, studies on *Moringa oleifera*, *Paullinia cupana*, and *Cucumis prophetarum* demonstrate the viability of using plant extracts for green synthesis of AgNPs, exhibiting antimicrobial, antioxidant, and anticancer activities. These studies collectively affirm that phytochemical-rich plant extracts can serve as efficient reducing and capping agents in nanoparticle synthesis, offering a sustainable and eco-friendly alternative to chemical methods. Overall, this work demonstrates that *Prosopis juliflora* is not only a potent source of therapeutic phytochemicals but also an effective bio-template for the green synthesis of silver nanoparticles. The resulting PJ-AgNPs hold significant promise in biomedical applications, particularly as antibacterial agents, with potential extensions into anticancer and antioxidant therapies.

CONCLUSION

This study successfully demonstrated the phytochemical richness and antibacterial potential of *Prosopis juliflora* ethanol extract and its biogenically synthesized silver nanoparticles (PJ-AgNPs). The presence of diverse bioactive secondary metabolites including phenols, tannins, flavonoids, and glycosides was confirmed through qualitative screening, UV-Visible spectrophotometry,

FTIR, and GC-MS analysis. A total of 35 compounds were identified, with key constituents likely contributing to the biological activity and nanoparticle synthesis. The formation of PJ-AgNPs was visually confirmed by a distinct color change and further validated using spectroscopic and microscopic techniques. Characterization studies revealed the nanoparticles were spherical, with an average size of 70.21 nm and high elemental purity. Antibacterial assays revealed significant activity of both the ethanol extract and PJ-AgNPs against pathogenic bacteria. Notably, the extract showed maximum inhibition against *Enterococcus faecalis* (25 mm), while the PJ-AgNPs were most effective against *Salmonella typhi* (19 mm). Overall, the findings highlight *Prosopis juliflora* as a valuable source of phytochemicals and its potential in the green synthesis of silver nanoparticles with promising antimicrobial applications. This lays a foundation for future studies focused on optimizing nanoparticle production and exploring broader biomedical applications.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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