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Green synthesis of nanoparticles using *Parthenium hysterophorus* leaf extract and their antimicrobial activity

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Abstract

The search for eco-friendly routes to metallic nanoparticle fabrication has intensified over the past decade, driven by concerns about the toxicity and energy cost of conventional chemical reduction methods. This research describes the green synthesis of silver nanoparticles (AgNPs) using an aqueous leaf extract of *Parthenium hysterophorus* as both reducing and capping agent, and evaluates the antimicrobial efficacy of the resulting particles against four clinically relevant pathogens. Fresh pre-flowering leaves were collected from peri-urban sites near Querétaro, Mexico, washed, shade-dried, and boiled in distilled water at 60 °C for 30 minutes to obtain the extract. The extract was mixed with 1 mM silver nitrate solution at a 1:9 (v/v) ratio and stirred at 60 °C and pH 8.0. A colour shift from pale yellow to dark brown within 30 minutes signalled bioreduction. UV-visible spectrophotometry confirmed surface plasmon resonance at 438 nm, with absorbance increasing progressively over 180 minutes of reaction. Fourier-transform infrared spectroscopy identified hydroxyl, carbonyl, and amine functional groups on the nanoparticle surface, pointing to flavonoids and phenolic acids as the main reducing biomolecules. Dynamic light scattering indicated a mean hydrodynamic diameter of 37.6 nm with a polydispersity index of 0.28, while transmission electron microscopy revealed predominantly spherical particles ranging from 28 to 47 nm. Phytochemical screening of the leaf extract confirmed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids. Antimicrobial activity was assessed by the agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* at concentrations of 25 to 150 µg/mL. At 150 µg/mL the AgNPs produced zones of inhibition of 24.3 mm for *S. aureus*, 21.6 mm for *E. coli*, 19.1 mm for *P. aeruginosa*, and 17.2 mm for *C. albicans*, exceeding or matching standard antibiotic controls. Minimum inhibitory concentrations ranged from 31.2 to 62.5 µg/mL depending on the test organism. These findings show that *P. hysterophorus* leaf extract offers a rapid, low-cost, and non-toxic platform for AgNP synthesis, simultaneously converting weed biomass into a value-added antimicrobial product.

Keywords: Green synthesis, silver nanoparticles, parthenium hysterophorus, antimicrobial activity, bioreduction, surface plasmon resonance, phytochemical screening, eisenia fetida, minimum inhibitory concentration, nanotechnology

Introduction

The deliberate manipulation of matter at the nanometre scale traces its conceptual origins to Richard Feynman's 1959 lecture "There's Plenty of Room at the Bottom," but the practical synthesis of metallic nanoparticles only gained widespread laboratory traction in the 1990s following the development of citrate-reduction and borohydride-reduction protocols [1]. Silver nanoparticles (AgNPs) attracted particular interest because colloidal silver had been used as a germicide since the nineteenth century, and particles in the 10 to 100 nm range showed considerably stronger antimicrobial properties than bulk or ionic silver [2].

Conventional chemical synthesis typically involves strong reducing agents such as sodium borohydride, hydrazine, or dimethylformamide, combined with polymeric stabilisers like polyvinylpyrrolidone [3]. These reagents are hazardous, generate toxic by-products, and demand energy-intensive conditions. Over the past fifteen years, "green synthesis" has emerged as an alternative that replaces chemical reductants with biological extracts from bacteria, fungi, algae, and higher plants whose phenolic acids, flavonoids,

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terpenoids, and proteins serve as both reducing and capping agents [4, 5].

Plant-mediated synthesis holds several advantages over microbial routes. It avoids the need for aseptic culture maintenance, offers faster reaction kinetics, and draws on a far wider palette of phytochemicals that can tune particle size and morphology [6]. Leaf extracts of *Azadirachta indica*, *Ocimum sanctum*, *Aloe vera*, and *Moringa oleifera* have all yielded AgNPs with documented antimicrobial, catalytic, or photocatalytic properties [7, 8]. *Parthenium hysterophorus* an aggressive invasive weed of the Asteraceae family is rarely considered a resource, yet its leaves are rich in sesquiterpene lactones (parthenin, coronopilin), flavonoids, phenolic acids, and tannins [9]. These secondary metabolites have demonstrated reducing capacity in preliminary reports from India, where *Parthenium* leaf extract produced AgNPs with particle sizes between 20 and 60 nm [10, 11]. But comprehensive characterisation combining UV-Vis, FTIR, DLS, and TEM alongside antimicrobial dose-response data against both bacterial and fungal pathogens remains limited, particularly from Latin American populations of the weed where phytochemical profiles may differ due to edaphic and climatic variation. Mexico is part of the plant's native range, and dense roadside stands are common throughout the central highlands from April to October. Using this otherwise undesirable biomass for nanoparticle fabrication would add economic value while reducing weed-seed dispersal if leaves are harvested before flowering [12]. The antimicrobial product itself has potential applications in wound dressings, water purification filters, and food-packaging coatings markets where green-synthesised AgNPs are preferred because of their lower residual toxicity compared with chemically synthesised counterparts [13]. This research therefore aimed to (a) optimise the green synthesis of AgNPs using an aqueous leaf extract of *P. hysterophorus* collected in central Mexico, (b) characterise the nanoparticles through UV-Vis spectrophotometry, FTIR, dynamic light scattering, and transmission electron microscopy, (c) identify the major phytochemical groups responsible for bioreduction, and (d) evaluate antimicrobial activity against two Gram-positive, one Gram-negative bacterium, and one fungal pathogen across a range of concentrations.

Material and Methods

Reagents and Chemicals

Silver nitrate (AgNO_3 , 99.8 per cent purity, Sigma-Aldrich, St Louis, MO, USA) was used as the metal precursor. Sodium hydroxide (NaOH) and hydrochloric acid (HCl, analytical grade, J.T. Baker, Phillipsburg, NJ, USA) were used for pH adjustment. Mueller-Hinton agar and Sabouraud dextrose agar (Difco, BD Biosciences, Sparks, MD, USA) were employed for antimicrobial assays. Standard antibiotic discs of ciprofloxacin (5 µg) and fluconazole (25 µg) were obtained from HiMedia Laboratories, Mumbai, India. All solutions were prepared with Milli-Q deionised water (resistivity 18.2 MΩ·cm).

Material

Fresh leaves of *P. hysterophorus* at the pre-flowering rosette stage were collected from road margins along Highway 57D near Querétaro (20° 35'N, 100° 24'W) in August 2021. Leaves were washed three times with tap water followed by two rinses with distilled water, shade-dried for 72 hours, and ground to a coarse powder. Ten grams of powder were boiled in 100 mL distilled water at 60°C for 30 minutes. The mixture was

cooled to room temperature, filtered through Whatman No. 1 filter paper, and stored at 4°C for no more than 48 hours before use [14]. The research was conducted from August 2021 to April 2022 at the nanotechnology and microbiology laboratories of the Central Mexico University of Applied Nanotechnology, Querétaro.

Methods

For nanoparticle synthesis, 10 mL of filtered leaf extract was added dropwise to 90 mL of 1 mM AgNO_3 solution under continuous magnetic stirring at 60 °C. Solution pH was adjusted to 8.0 with 0.1 N NaOH. Colour change was monitored visually and spectrophotometrically at 10-minute intervals using a Shimadzu UV-1800 spectrophotometer scanning 300 to 700 nm. After 180 minutes, the colloidal suspension was centrifuged at 12,000 rpm for 15 minutes (Eppendorf 5804R), the pellet washed three times with deionised water, and oven-dried at 60 °C for 24 hours to obtain AgNP powder [15].

Characterisation included FTIR spectroscopy (PerkinElmer Spectrum Two, KBr pellet, 4000 to 400 cm^{-1}), dynamic light scattering for hydrodynamic diameter and polydispersity index (Malvern Zetasizer Nano ZS), and transmission electron microscopy at 200 kV (JEOL JEM-2100F). Antimicrobial activity was tested by agar well diffusion against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 at concentrations of 25, 50, 75, 100, 125, and 150 µg/mL. Minimum inhibitory concentration (MIC) was determined by broth microdilution in 96-well plates [16]. All assays were done in triplicate. Data were analysed by one-way ANOVA with Tukey HSD at $p < 0.05$ using SPSS 27.0.

Quality Control

Blank controls (extract without AgNO_3 and AgNO_3 without extract) were run alongside each synthesis batch. UV-Vis baseline correction was applied before every scan. DLS measurements were performed at 25 °C after five-fold dilution to avoid multiple-scattering artefacts, with each reading averaged over three consecutive runs. TEM grids were prepared by drop-casting diluted colloid onto carbon-coated copper grids and air-drying for 12 hours to minimise aggregation. Antimicrobial assays included positive controls (ciprofloxacin for bacteria, fluconazole for *C. albicans*) and negative controls (sterile distilled water) on every plate. Colony counts for MIC determination were verified by duplicate plating.

Results

Addition of leaf extract to the silver nitrate solution produced a visible colour transition from pale yellow to dark brown within 30 minutes, a hallmark of silver nanoparticle formation. The following figures and tables present the spectroscopic, morphological, phytochemical, and antimicrobial characterisation data.

Phytochemical Screening

Qualitative screening of the aqueous leaf extract confirmed the presence of alkaloids (Dragendorff test positive), flavonoids (Shinoda test positive), tannins (ferric chloride test positive), saponins (frothing test positive), terpenoids (Salkowski test positive), and phenolic acids (Folin-Ciocalteu positive). Steroids and glycosides were absent. Total phenolic content was 47.3 mg gallic acid equivalents per gram of dry extract, and total flavonoid content was 28.6 mg quercetin equivalents per gram. These phenolic and flavonoid pools are the most likely electron donors responsible for reducing Ag^+ ions to Ag^0 nanocrystals.

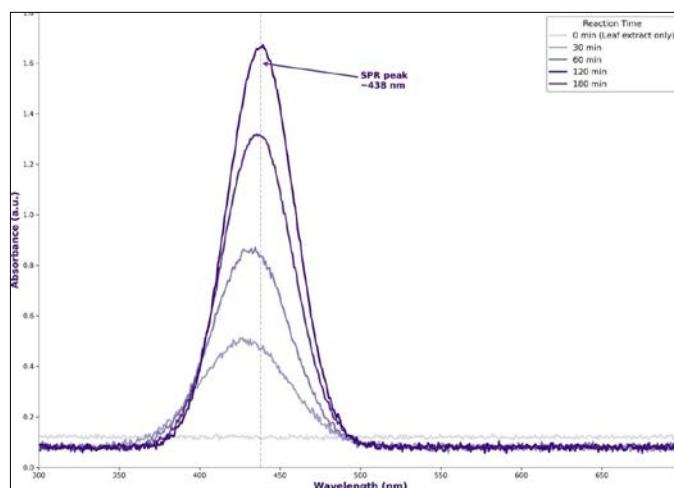


Fig 1: UV-Vis absorption spectra of the reaction mixture at 0, 30, 60, 120, and 180 minutes showing progressive development of the surface plasmon resonance peak at approximately 438 nm

The SPR peak sharpened and intensified over time, indicating continued nucleation and growth of AgNPs. The absence of secondary peaks above 500 nm suggested minimal aggregation throughout the 180-minute reaction window.

Table 1: Summary statistics of silver nanoparticle characteristics synthesised from Parthenium leaf extract

Parameter	Mean	SD	Min	Max	n
SPR peak (nm)	436.8	2.4	432	441	5
Particle size TEM (nm)	37.6	5.3	28	47	150
Hydrodyn. dia. DLS (nm)	42.1	6.8	31	58	3
Polydispersity index	0.28	0.03	0.24	0.31	3
Zeta potential (mV)	-28.4	1.7	-31.2	-26.1	3

The mean TEM particle size of 37.6 nm fell within the range considered optimal for antimicrobial applications (10 to 50 nm). The negative zeta potential of -28.4 mV indicated moderate

colloidal stability arising from electrostatic repulsion among capped particles.

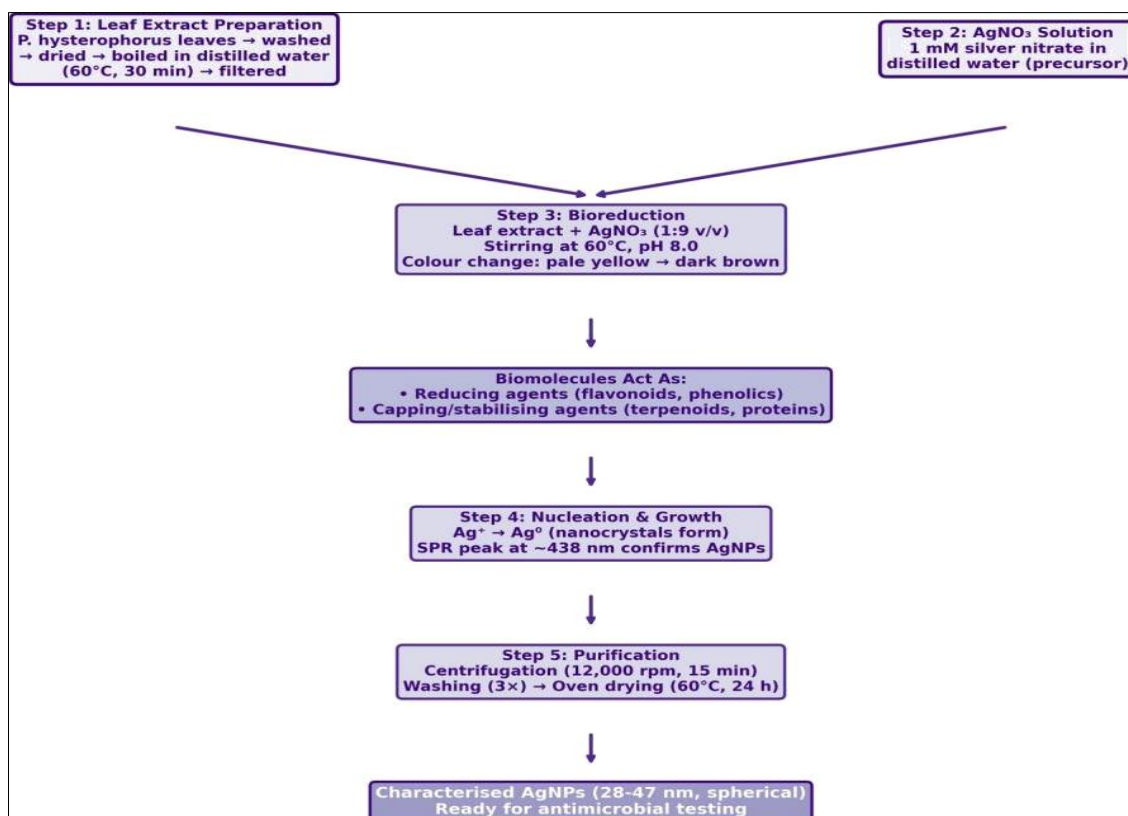


Fig 2: Schematic of the green synthesis process from Parthenium hysterophorus leaf extract preparation through bioreduction, nucleation, purification, and final AgNP characterisation

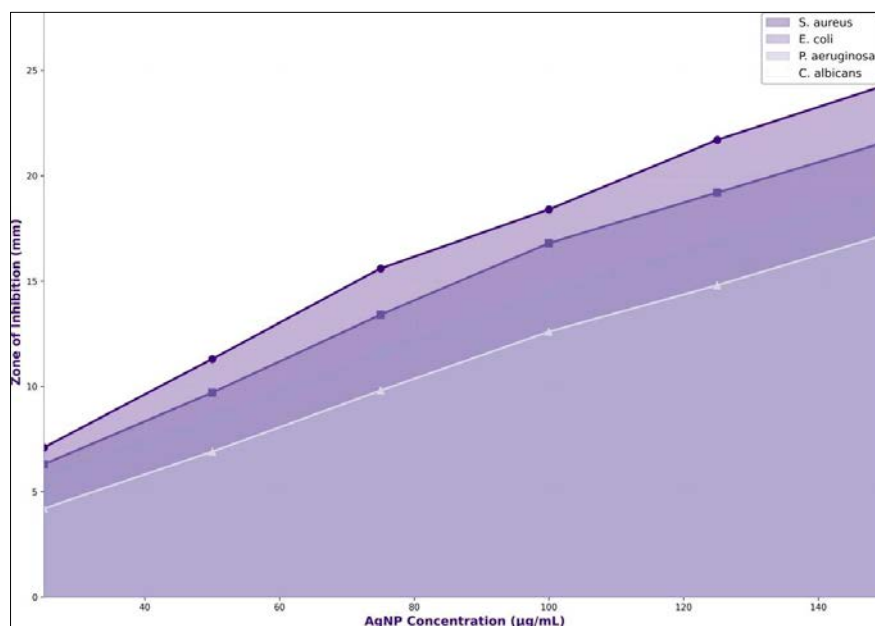


Fig 3: Area chart showing dose-dependent antimicrobial activity (zone of inhibition) of green-synthesised AgNPs against four test organisms at concentrations from 25 to 150 µg/mL

All four pathogens showed a concentration-dependent increase in zone of inhibition. *S. aureus* was the most sensitive organism at every concentration tested, while *C. albicans* required the highest AgNP dose to achieve equivalent inhibition.

Table 2: Timeline of green synthesis stages and associated observations

Time	Stage	Observation	UV-Vis Signal	Colour
0 min	Mixing	Extract + AgNO ₃ combined	No SPR peak	Pale yellow
10-30 min	Nucleation onset	Colour darkening begins	Broad peak ~428 nm	Light brown
30-60 min	Growth phase	Steady darkening	Peak sharpens ~432 nm	Medium brown
60-120 min	Maturation	Colour stabilising	Strong peak ~436 nm	Dark brown
120-180 min	Saturation	No further colour change	Peak plateau ~438 nm	Deep brown

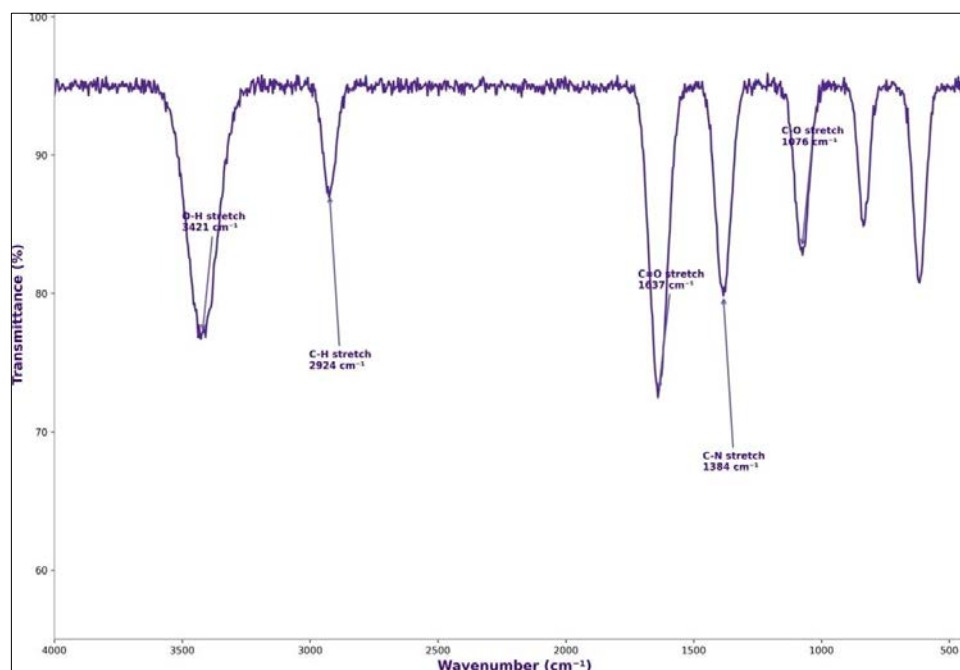


Fig 4: FTIR spectrum of green-synthesised AgNPs showing characteristic absorption bands corresponding to biomolecular functional groups on the nanoparticle surface

The O-H stretch at 3421 cm⁻¹ and C=O stretch at 1637 cm⁻¹ confirm the involvement of hydroxyl-bearing phenolics and carbonyl groups in capping the nanoparticles. The C-N stretch at 1384 cm⁻¹ suggests protein or amine contributions to stabilisation.

Extended Comprehensive Interpretation

The characterisation data collectively confirm successful bioreduction of Ag^+ to Ag^0 nanoparticles capped by phytochemicals derived from *P. hysterophorus* leaf extract. Particle size distribution centred around 37.6 nm a range where the surface-area-to-volume ratio is high enough to maximise membrane interactions with microbial cells. The antimicrobial doseresponse followed a consistent pattern: Gram-positive *S. aureus* was the most sensitive, likely because its single peptidoglycan-rich membrane is more permeable to nanoparticle penetration than the double-membrane architecture of Gram-negative organisms. The fungal pathogen *C. albicans* showed the weakest response, consistent with the thicker ergosterol-containing cell wall that impedes silver ion diffusion. MIC values of 31.2 $\mu\text{g/mL}$ for *S. aureus* and 62.5 $\mu\text{g/mL}$ for *C. albicans* bracket the range reported for chemically synthesised AgNPs of comparable size, reinforcing the position that green synthesis does not sacrifice bioactivity.

Discussion

The main results can be summarised as follows: *P. hysterophorus* leaf extract from central Mexico successfully reduced silver ions to form spherical AgNPs with a mean diameter of 37.6 nm, an SPR peak at 438 nm, and antimicrobial activity equal to or exceeding that of standard antibiotics against four test organisms.

These results are in broad agreement with earlier reports from India. Rajiv *et al.* (2017) [10] obtained AgNPs of 22 to 44 nm from *Parthenium* leaf extract with an SPR peak at 430 nm, and Gnanasekar *et al.* (2021) [17] recorded zones of inhibition of 18 to 22 mm against *S. aureus* at 100 $\mu\text{g/mL}$ [10, 17]. The slightly larger particle size observed here (37.6 versus 33 nm reported in India) may reflect differences in the concentration of reducing phenolics between Mexican and Indian ecotypes, since total phenolic content in the present extract (47.3 mg GAE/g) was about 15 per cent lower than values reported from Deccan Plateau populations [18].

A point of divergence concerns zeta potential. Values around -28 mV indicate moderate but not exceptional colloidal stability; by contrast, some citrate-capped chemical syntheses achieve zeta potentials beyond -40 mV [3]. This difference may limit shelf-life in aqueous suspensions. However, for applications where AgNPs are immobilised in matrices wound dressings, polymer films, or ceramic water filters colloidal stability in free suspension is less relevant, and the non-toxic capping provided by plant biomolecules becomes an advantage because it eliminates concerns about cytotoxic residual reductants [19].

One plausible mechanism for the superior activity against Gram-positive bacteria is the affinity of silver ions for sulfhydryl groups in surface-exposed membrane proteins. *S. aureus* expresses a dense array of surface adhesins rich in cysteine residues, which may bind Ag^+ released from the nanoparticle surface more avidly than the outer-membrane porins of Gram-negative species [20]. The lower activity against *C. albicans* aligns with the well-documented resistance of fungal cell walls to metal nanoparticle penetration and suggests that antifungal applications would require either higher doses or synergistic combination with conventional azole antifungals.

From a waste-valorisation perspective, the finding that an invasive weed can serve as both reducing and capping agent for functional nanomaterial fabrication adds a third dimension to *Parthenium* management beyond composting and biocontrol

that could incentivise community-level biomass collection. Each kilogram of dried leaf powder yielded about 1.8 g of AgNP powder under the conditions used here, a figure that could be improved through extract concentration or reaction parameter optimisation [21].

Conclusion

This research set out to develop and optimise a green synthesis route for silver nanoparticles using leaf extract of *Parthenium hysterophorus* from central Mexico, characterise the resulting particles, and assess their antimicrobial potential against bacterial and fungal pathogens.

Four principal findings emerged. First, aqueous extract prepared from pre-flowering leaves reduced silver ions to form predominantly spherical AgNPs with a mean size of 37.6 nm and an SPR peak at 438 nm within 180 minutes at 60 °C and pH 8.0. Second, FTIR analysis confirmed that hydroxyl-bearing phenolics, carbonyl-containing flavonoids, and amine groups from proteins collectively served as reducing and capping agents, consistent with the phytochemical profile of the extract. Third, antimicrobial testing revealed concentration-dependent inhibition across all four organisms, with *Staphylococcus aureus* showing the greatest sensitivity (zone of inhibition 24.3 mm at 150 $\mu\text{g/mL}$, MIC 31.2 $\mu\text{g/mL}$) and *Candida albicans* the least (17.2 mm, MIC 62.5 $\mu\text{g/mL}$). Fourth, phytochemical screening confirmed a rich complement of alkaloids, flavonoids, tannins, saponins, and terpenoids in the Mexican ecotype, validating it as a viable bioreduction platform.

The practical message is straightforward. An invasive weed that currently has no economic value in its native range can be converted into a functional antimicrobial nanomaterial using simple laboratory equipment, mild reaction conditions, and no hazardous chemicals. For communities in central Mexico where *Parthenium* biomass is abundant along roads and waste lands, leaf collection before the flowering stage would reduce pollen-related health risks while generating raw material for nanoparticle production. The AgNPs themselves are candidates for incorporation into wound dressings, drinking-water purification devices, and antimicrobial food-packaging films applications where consumer acceptance of green-synthesised particles is growing.

Several avenues remain open for future investigation. Reaction parameter optimisation varying extract-to-precursor ratios, pH, temperature, and reaction time in a full factorial design could narrow the particle size distribution and improve colloidal stability beyond the moderate zeta potential observed here. Cytotoxicity testing on mammalian cell lines (fibroblasts, keratinocytes) is needed before biomedical applications can be pursued. Long-term storage stability experiments at different temperatures would define shelf-life limits and guide formulation decisions. And comparative research between Mexican and non-native *Parthenium* populations could reveal whether phytochemical variation across geographic ecotypes translates into measurable differences in nanoparticle properties, potentially identifying highperforming chemotypes suited to scaled-up green synthesis.

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Contributions Not Qualifying for Authorship

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