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Research Article

Biosynthesis of Silver Nanoparticles using leaf Extracts of *Tridax procumbens*

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Article Info

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Abstract

In the present study, silver nanoparticles (AgNPs) were biosynthesized using aqueous leaf extracts of *Tridax procumbens* and evaluated for their antibacterial potential. The UV–Visible absorption spectrum of the synthesized AgNPs exhibited a characteristic surface plasmon resonance (SPR) peak at 417 nm, confirming nanoparticle formation. The minimum inhibitory concentration (MIC) of the biosynthesized AgNPs against *Escherichia coli*, a common plant pathogenic bacterium, was determined to be 0.450 mg/mL. Antibacterial assays revealed significant inhibitory activity, with an inhibition zone measuring 22 mm in diameter. These results demonstrate that *Tridax procumbens*-derived AgNPs possess promising antibacterial properties and can serve as potential biogenic antimicrobial agents.

Keywords: Silver nanoparticles, AGNPs, bark extract, UV analysis, *Tridax procumbens*, *Escherichia coli*.

Introduction

The green synthesis of nanoparticles has gained considerable attention as a sustainable and eco-friendly alternative to conventional physical and chemical methods. Metals such as silver, gold, iron, zinc, copper, platinum, palladium, and selenium are commonly utilized in nanoparticle synthesis due to their distinctive biological and chemical properties. Among these, silver is particularly noteworthy for its potent antifungal

and antibacterial activities [1]. The pioneering work of Gardea-Torresdey *et al.* [4], who employed *Medicago sativa* (alfalfa) sprouts for the biosynthesis of silver nanoparticles (AgNPs), marked a significant milestone in the field of green nanotechnology.

Green synthesis of silver nanoparticles is an eco-conscious approach that employs biological resources such as plant extracts, microorganisms, and natural polymers as both reducing and stabilizing agents. Unlike conventional physical and chemical methods, which often rely on toxic reagents, high energy input, and generate hazardous by-products, green synthesis is safe, cost-effective, and environmentally benign [14,,1]. In this process, silver ions (Ag^{+}) derived from precursors such as silver nitrate are reduced to metallic silver (Ag^{0}) by phytochemicals present in plant extracts. These biomolecules—flavonoids, terpenoids, phenolics, proteins, and enzymes—act synergistically as reducing and capping agents, influencing the nucleation, growth, morphology, and stability of the nanoparticles. Among various biological routes, plant-mediated synthesis is especially advantageous due to its simplicity, rapidity, scalability, and elimination of microbial culture maintenance, making it suitable for large-scale nanoparticle production.

Biosynthesized silver nanoparticles have garnered wide interest for their broad-spectrum antimicrobial efficacy, biocompatibility, and applications across diverse sectors such as medicine, environmental remediation, food packaging, and textiles. The emergence of antibiotic-resistant bacterial strains has further underscored the need for novel antimicrobial agents, and metal nanoparticles offer a promising solution [3]. As a result, green synthesis of AgNPs aligns well with the principles of green chemistry and sustainable development.

In the present study, AgNPs were synthesized using aqueous leaf extracts of *Tridax procumbens* Linn., a medicinally important plant belonging to the family Asteraceae. Commonly known as “coat buttons” in English and “Ghamra” in Hindi, *T. procumbens* is a fast-growing, prostrate or semi-erect herb widely distributed across tropical, subtropical, and temperate regions, including India. The plant is characterized by hairy stems, white-rayed flowers with yellow centers, and oppositely arranged serrated leaves. Although often considered a weed, *T. procumbens* holds substantial ethnomedicinal significance and is widely used in traditional medicine systems, including Ayurveda, for its wound-healing, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, hepatoprotective, and hypotensive properties. The leaves and flowers are rich in bioactive phytochemicals such as flavonoids, terpenoids, and phenolic acids, which contribute to its pharmacological potential [13]. It has also been employed to treat ailments such as malaria, dysentery, diarrhea, cough with mucus, hair loss, and stomach disorders. Moreover, oleanolic acid isolated from *T. procumbens* has shown potent antidiabetic activity through α-glucosidase inhibition [9].

Recently, *Tridax procumbens* has emerged as a promising candidate in nanotechnology for the green synthesis of metallic nanoparticles, owing to its rich phytochemical composition that facilitates nanoparticle formation and stabilization. Hence, the present work focuses on the biosynthesis of silver nanoparticles using aqueous leaf extracts of *Tridax procumbens* and their evaluation for antibacterial activity.

Material And Methods

Collection of Plant and preparation of aqueous extract

Fresh and healthy leaves of *Tridax procumbens* were collected from the campus of Sharadchandra College, Naigaon, District Nanded, Maharashtra, India. The collected leaves were thoroughly washed first with tap water and subsequently with distilled water to remove dust, debris, and other surface contaminants. The cleaned leaves were then cut into small pieces and air-dried at room temperature until complete moisture removal.

Approximately 10 g of the dried leaf material was weighed and transferred into a 250 mL

beaker containing 100 mL of distilled water. The mixture was boiled for 20 minutes to facilitate the extraction of phytochemicals. After boiling, the extract was filtered three times using Whatman No. 1 filter paper to remove particulate matter and obtain a clear aqueous solution. The filtrate was stored in sterile 250 mL conical flasks at 4°C until further use for silver nanoparticle (AgNP) synthesis [10]. Metal nanoparticles have attained a great importance due to their unique feature such as catalytic, magnetic, optical and electrical properties[15,16]. Silver nanoparticles (AgNPs) have attracted considerable interest in biological studies because of their ease of preparation, good biocompatibility, and relatively large surface area. The synthesis of nanoparticles of different chemical compositions, sizes, and controlled monodispersity is an important area of research in nanotechnology [17]. A variety of chemical approaches have also been utilized to produce silver nanoparticles with different size distribution and different shapes [18,19]. Silver nanoparticles have received considerable attention as antimicrobial agents and have been shown to be an effective antimicrobial agent [20].

Throughout all experimental procedures, strict aseptic conditions were maintained to prevent contamination and ensure the accuracy and reliability of the results.

Preparation of 0.01 M Silver Nitrate Solution

A 0.01 M silver nitrate ($AgNO_3$) solution was prepared by dissolving 1.67 g of analytical-grade $AgNO_3$ in distilled water. The solution was prepared in a volumetric flask under aseptic conditions using an analytical balance to ensure precision in measurement. The prepared solution was stored in an amber bottle to prevent photodegradation until further use.

Synthesis of Silver Nanoparticles

For the green synthesis of silver nanoparticles (AgNPs), 100 mL of *Tridax procumbens* leaf extract was mixed with 200 mL of 0.01 M $AgNO_3$ solution. The reaction mixture was subjected to continuous stirring and heating at 80 °C to promote the reduction of silver ions. Subsequently, the mixture was exposed to microwave irradiation to achieve complete bioreduction.

A distinct color transformation from pale yellow to yellowish brown, reddish brown, and finally to colloidal brown was observed, indicating the formation of AgNPs [6]. The reduction of Ag^+ ions to Ag^0 nanoparticles was monitored using UV–Visible spectroscopy, which confirmed the characteristic surface plasmon resonance (SPR) of silver nanoparticles. To prevent photoactivation of silver nitrate, the reaction was performed in the dark at room temperature. The resulting colloidal suspension was sealed and stored for further analysis.

Scanning Electron Microscopy (SEM) Analysis

For morphological characterization, 10 mL of the biosynthesized AgNP suspension was centrifuged at 4000 rpm for 12 minutes. The resulting pellet was collected on a clean watch glass and dried completely at 150 °C. The dried sample was redispersed in 0.2 mL of distilled water and transferred into a sterile microcentrifuge tube. To prevent light-induced degradation, the samples were wrapped in aluminum foil and stored until analysis. The surface morphology and particle size of the synthesized AgNPs were examined using a Scanning Electron Microscope (SEM).

Assessment of Antimicrobial Activity

The antibacterial potential of biosynthesized silver nanoparticles (AgNPs) against *Escherichia coli* was evaluated using the agar well diffusion method as described by Perez *et al.*[11]. The bacterial culture was first inoculated into nutrient broth and incubated overnight in a shaker incubator at 37 °C. Sterile nutrient agar plates were prepared and allowed to solidify, after which three wells of 6 mm diameter were created in each plate using a sterile cork borer. The wells were loaded with 20 µL of AgNP solution, 25 µg/mL ampicillin (positive control), and 20 µL of *Tridax procumbens* leaf extract (negative control), respectively. The plates were incubated at 37 °C for 24 hours, and the antibacterial activity was

assessed by measuring the diameter of inhibition zones in millimeters.

The Minimum Inhibitory Concentration (MIC) of the biosynthesized AgNPs was determined using different concentrations (0.350, 0.450, 0.550, and 0.650 mg/mL) prepared in 1 mL of acetone. Nutrient agar medium was sterilized, poured into Petri plates, and allowed to solidify. Bacterial cultures were spread on the surface of the solidified agar, and wells of 6 mm diameter were made. Each well was loaded with 20 µL of the respective AgNP concentration. Plates were incubated at 37 °C for 24 hours, and the inhibition zones were observed and measured in millimeters. The lowest concentration of AgNPs that produced a clear zone of inhibition was recorded as the MIC. All experiments were performed in triplicate to ensure accuracy and reproducibility.

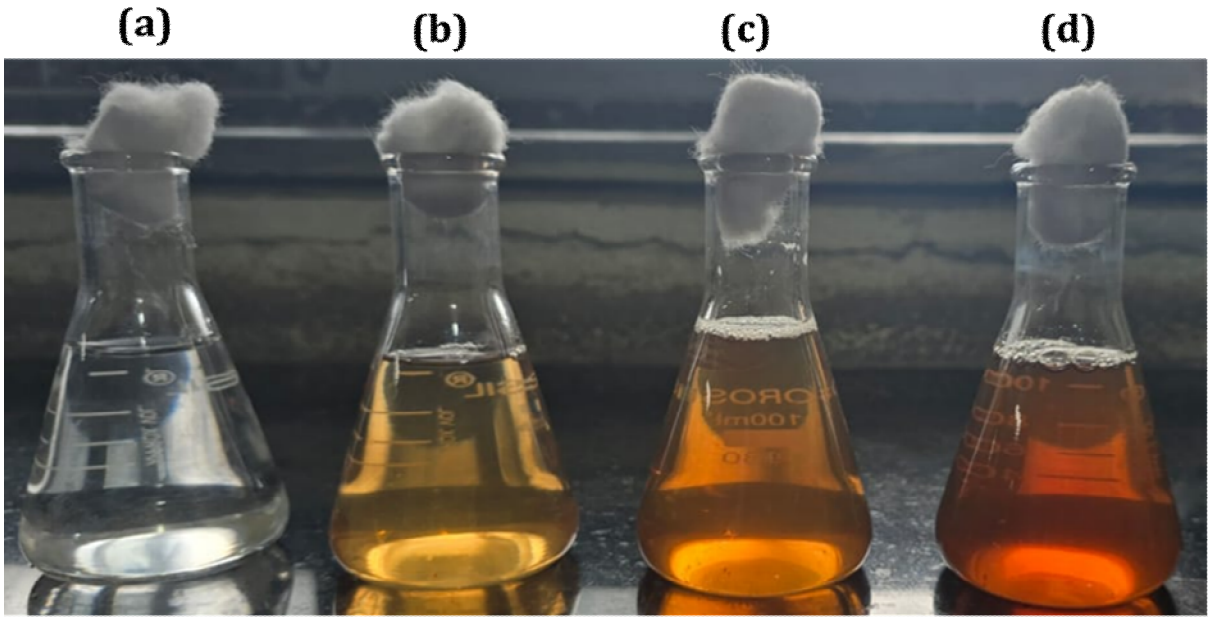

Fig 1: Biosynthesis of AGNPs using *Tridax procumbens* leaf extract (a) silver nitrate sol^{n},(b) AGNPs after 30 min, (c) AGNPs after 60 min and (d) AGNPs after 150 min).

Results and Discussion

Visual Examination

The synthesis of silver nanoparticles (AgNPs) was initially confirmed through visual observation of the reaction mixture comprising $AgNO_3$ solution and *Tridax procumbens* leaf extract. As shown in Figure 1, a noticeable color change occurred over time. Within 15 minutes, the colorless solution began to turn faint yellow, indicating the initial formation of AgNPs. With increasing reaction time, the solution gradually darkened to brown at 150 minutes, reflecting a higher concentration and growth of silver nanoparticles [2]. No significant change in color was observed beyond 150 minutes, suggesting the completion of the bioreduction process and stabilization of the nanoparticles.

UV–Visible Spectroscopic Analysis

The formation and reduction of silver nanoparticles were further confirmed using UV–Visible spectrophotometry. The biosynthesized AgNPs exhibited a characteristic absorption peak at 418 nm, corresponding to the surface plasmon resonance (SPR) of silver nanoparticles. This peak is consistent with the presence of stable AgNPs synthesized via the phytochemicals in *Tridax procumbens* leaf extract, demonstrating the effectiveness of the green synthesis approach.

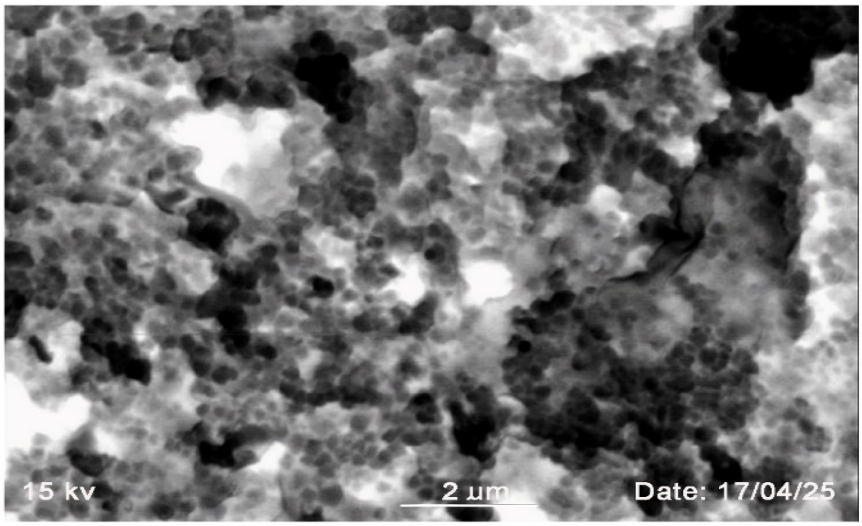

Fig. 2: Scanning electron microscopy (SEM) image showing the morphology of biosynthesized silver nanoparticles (AgNPs).

Morphological Analysis

Scanning Electron Microscopy (SEM) analysis revealed that the biosynthesized AgNPs were predominantly small, with shapes ranging from spherical to square. The nanoparticles were observed to exist in an aggregated form, suggesting partial agglomeration during the synthesis process (Figure 2). The uniform morphology and nanoscale size of the particles indicate effective reduction and stabilization by the phytochemicals present in *Tridax procumbens* leaf extract.

Table 1: MIC of Ag NPs against *Escherichia coli*

	MIC against *Escherichia coli.*			
Conc AGNPs	0.350 mg/ml	0.450 mg/ml	0.550mg/ml	0.650 mg/ml
Zone of Inhibition	-	+	+	+

Positive (+): Indicating Zone of Inhibition; Negative (–): Indicating No Zone of Inhibition

Minimum Inhibitory Concentration (MIC) and Antibacterial Activity

The antibacterial efficacy of biosynthesized silver nanoparticles (AgNPs) against *Escherichia coli* was further evaluated by determining the Minimum Inhibitory Concentration (MIC) and using the agar well diffusion method. Increasing concentrations of AgNPs resulted in a corresponding delay in bacterial growth, indicating a dose-dependent inhibitory effect (Table 1). No inhibition was observed at 0.350 mg/mL. However, at concentrations of 0.450, 0.550, and 0.650 mg/mL, complete inhibition of *E. coli* growth was achieved, with a slight increase in the zone of inhibition at higher concentrations. These observations indicate that the MIC of biosynthesized AgNPs against *E. coli* is 0.450 mg/mL, as this was the lowest concentration at which bacterial growth was effectively inhibited. Comparable studies, such as Shinde and Patil [12], reported MIC values of 0.060 mg/mL for biosynthesized AgNPs against *Xanthomonas campestris* pv. *malvacearum*, a plant pathogenic bacterium.

The antibacterial activity, assessed using the agar well diffusion method, demonstrated that biosynthesized AgNPs exhibited significant inhibitory effects against *E. coli*. At the MIC concentration of 0.450 mg/mL, AgNPs produced a zone of inhibition measuring 22 mm in diameter, which was greater than that of the conventional antibiotic ampicillin (15 mm) and the leaf extract alone (10 mm). These results confirm the potent antibacterial potential of AgNPs synthesized from *Tridax procumbens* and are consistent with previous reports on the antimicrobial efficacy of green-synthesized silver nanoparticles [5,7,8].

Conclusions

In this study, silver nanoparticles (AgNPs) were successfully synthesized using aqueous leaf extracts of *Tridax procumbens* through a green, eco-friendly, and cost-effective approach. The formation of AgNPs was confirmed by visual color change, UV–Visible spectroscopy, and Scanning Electron Microscopy, which revealed predominantly spherical to square-shaped nanoparticles with partial agglomeration. The biosynthesized AgNPs demonstrated significant antibacterial activity against *Escherichia coli*, with a minimum inhibitory concentration (MIC) of 0.450 mg/mL and a maximum inhibition zone of 22 mm, surpassing the activity of both the plant extract alone and the standard antibiotic control.

These findings highlight the potential of *Tridax procumbens*-mediated AgNPs as a promising biogenic antimicrobial agent. The study reinforces the value of plant-mediated nanoparticle synthesis as a sustainable alternative to conventional chemical methods, with potential applications in medicine, agriculture, and environmental biotechnology. Future studies could focus on exploring the mechanism of antimicrobial action, cytotoxicity assessment, and potential large-scale applications of these biosynthesized nanoparticles.

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