

DEVELOPMENT OF *TRIDAX PROCUMBENS*-MEDIATED SILVER NANOPARTICLES AND THEIR ANTIOXIDANT PROPERTIES

SOLOMON E. SHAIBU^{1,2*}, RUTH E. OKON¹, NNAMSO D. IBUOTENANG³ AND IDONGESIT B. ANWETING¹

1 Department of Chemistry, Faculty of Physical Sciences, University of Uyo, Uyo, Akwa Ibom State, NIGERIA

2 International Center for Energy and Environmental Sustainability Research (ICEESR), University of Uyo, Uyo, Akwa Ibom State, NIGERIA

3 Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, NIGERIA

Plant-mediated synthesis of metal nanoparticles provides a sustainable alternative to conventional chemical routes. This study reports the green synthesis of silver nanoparticles (AgNPs) using *Tridax procumbens* root extract as a source of reducing and stabilizing phytochemicals and evaluates their antioxidant activity. Qualitative phytochemical screening confirmed the presence of different phytochemicals. FTIR, XRD, SEM, TEM, and EDX analyses confirmed the formation of crystalline, predominantly spherical, silver-rich nanoparticles with some agglomeration. Antioxidant activity was evaluated using the DPPH free-radical scavenging assay. The AgNPs exhibited moderate, concentration-dependent activity, with inhibition increasing from 28.6% at 25 µg/mL to 61.8% at 200 µg/mL, compared with 18.4–46.2% for the crude root extract and 42.7–85.9% for ascorbic acid over the same concentration range. The estimated IC₅₀ values were 132.4 µg/mL for the AgNPs, 226.7 µg/mL for the crude extract, and 58.3 µg/mL for ascorbic acid. These findings indicate that *T. procumbens*-mediated AgNPs have measurable but moderate antioxidant activity.

Keywords: silver nanoparticles, *Tridax procumbens*, green synthesis, antioxidant activity, nanobiotechnology

1. Introduction

Nanotechnology has emerged as a transformative field across materials science, medicine, environmental engineering, and biotechnology due to the unique physicochemical properties of materials at the nanoscale (1–100 nm). Among nanomaterials, silver nanoparticles (AgNPs) have attracted considerable attention for their remarkable antimicrobial, anti-inflammatory, and antioxidant activities, as well as their potential applications in pharmaceuticals, food packaging, water purification, and diagnostics [1]–[3].

Traditionally, AgNPs have been synthesized using chemical and physical methods, including chemical reduction, photochemical reactions, and laser ablation [4]–[9]. Although these methods can produce well-defined nanoparticles, they often involve toxic chemicals such as sodium borohydride or hydrazine, high energy inputs, and environmentally hazardous by-products.

This not only increases production costs but also raises serious environmental and health concerns. These

limitations have led to the development of green synthesis methods that employ plant extracts and microorganisms, including bacteria, fungi, and algae, as natural sources of reducing and capping agents to synthesize nanoparticles in a more sustainable and biocompatible manner [10],[11]. Green synthesis of AgNPs offers several advantages, including cost-effectiveness, energy efficiency, and reduced environmental footprint. More importantly, bio-reduced nanoparticles often exhibit enhanced biological properties, attributed to phytochemicals, proteins, enzymes, and secondary metabolites derived from the biological sources used in their synthesis [12]. For instance, plant extracts such as *Tridax procumbens*, rich in polyphenols, flavonoids, alkaloids, and terpenoids, not only reduce silver ions (Ag⁺) to metallic silver (Ag⁰) but also serve as stabilizing agents, thereby improving the functional performance of the nanoparticles [13]–[16]. Among the various biological activities, the antioxidant properties of silver nanoparticles have received considerable attention, because of their potential biomedical applications. Oxidative stress, caused by an

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*Correspondence: shaibusolomon@uniuyo.edu.ng

imbalance between reactive oxygen species and antioxidant defenses, plays a pivotal role in the progression of chronic diseases, including cancer, neurodegenerative disorders, cardiovascular diseases, and diabetes. Therefore, materials that can mitigate oxidative stress by scavenging free radicals or modulating oxidative pathways are of therapeutic interest.

Previous studies on *Tridax procumbens*-mediated AgNPs have predominantly focused on leaf or aerial part extracts, which are rich in polyphenols and flavonoids and have yielded AgNPs with notable antioxidant and antibacterial activities. For example, Rani et al. and Baig et al. reported the green synthesis of AgNPs using *T. procumbens* leaves and demonstrated strong antibacterial activity [14],[17].

Similar studies employing leaf and flower extracts have confirmed that these plant parts can serve as efficient reducing and capping agents in AgNP synthesis [10,11,18]. By contrast, the root of *T. procumbens*, although known to contain bioactive phenolics, tannins, and alkaloids, has received very limited attention in the context of nanoparticle synthesis. This represents an important gap in current knowledge, as the phytochemical profile of *T. procumbens* roots remains largely uncharacterized relative to the extensively studied leaf extracts, and its influence on nanoparticle properties and biological activities has received little attention. Addressing this gap, the present work specifically targets the root extract of *T. procumbens* as a green synthesis platform for AgNPs and evaluates the antioxidant properties of the resulting nanomaterials.

Recent studies suggest that AgNPs synthesized using plant extracts possess significant free-radical scavenging capabilities. This effect has been attributed to bioactive compounds retained as reducing, capping and stabilizing agents on the nanoparticle surface adsorbed during green synthesis [7],[18]. For example, [18] reported that silver nanoparticles synthesized from *Azadirachta indica* and *Ocimum sanctum* showed antimicrobial activity against bacteria that cause urinary tract infections (UTIs). Tulsi-based nanoparticles were most effective against *S. aureus*, producing an inhibition zone of 23 mm, while neem-based nanoparticles showed moderate antibacterial activity, with greater inhibition observed at higher concentrations [19],[20].

This study aims to synthesize silver nanoparticles (AgNPs) using *Tridax procumbens* root extract as a natural reducing and stabilizing agent, and to investigate their physicochemical characteristics and antioxidant properties, with a view to their potential biomedical and environmental applications.

2. Methods and materials

2.1. Materials

The materials and equipment used in the study include *Tridax procumbens* root, sterile distilled water, a 250 mL beaker, Whatman filter paper No. 1, a kitchen grinder

(Ken Star, India), a magnetic stirrer (MS20D, Witeg Labortechnik, Germany), and centrifuge tubes. For the green synthesis of silver nanoparticles, 3 mM silver nitrate (AgNO_3) was used as the key precursor. The reagents used for the antioxidant assays included DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, ascorbic acid, β -carotene, linoleic acid, chloroform, Tween 80, and tert-butylhydroquinone (TBHQ).

2.2. Methods

2.2.1. Collection of plant material and extraction

The roots of *Tridax procumbens* were first washed thoroughly with tap water to remove surface dust, then rinsed with distilled water to remove residual impurities. 10 g of the cleaned roots were transferred into a 250 mL beaker containing 100 mL of sterile distilled water. The mixture was boiled for approximately 5 minutes and then allowed to cool naturally to room temperature (about 30 °C). After cooling, the boiled roots were homogenized using a mortar and pestle to facilitate the release of their bioactive compounds. The resulting paste was then filtered using Whatman No. 1 filter paper to separate solid residues and obtain a clear liquid extract. The filtrate, enriched with phytochemicals from the root, served as the biological medium for the green synthesis of silver nanoparticles.

2.2.2. Phytochemical screening of extract

The ethanolic extract was subjected to qualitative phytochemical screening standard chemical tests to detect the presence of alkaloids, flavonoids, glycosides, proteins, steroids, carbohydrates, phenolic compounds, tannins, terpenes, and terpenoids.

2.2.3. Green synthesis of AgNPs

A 10 mL aliquot of freshly prepared aqueous extract was mixed with 90 mL of a 3 mM silver nitrate solution, at a 1:9 ratio. This proportion was selected based on prior optimization studies and literature reports demonstrating efficient nanoparticle synthesis at this proportion [13],[16]. The mixture was placed on a magnetic stirrer equipped with a temperature-controlled ceramic-coated aluminum plate (Magnetic stirrer MS20D, Witeg Labortechnik, Wertheim, Germany) for nanoparticle synthesis. To facilitate the reaction, the mixture was stirred moderately (50 rpm) at 80 °C in the dark. The formation of silver nanoparticles (AgNPs) was confirmed by the appearance of a dark brown color.

2.2.4. Antioxidant activity

The technique outlined by [16] was used to assess the antioxidant activity of *T. procumbens* extract and manufactured AgNPs. The 3,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity test was used in the study to evaluate the antioxidant capacity.

2.2.5 Estimation of DPPH free radical scavenging activity

The DPPH free radical scavenging activity assay was employed to assess the antioxidant activity following the methodology described by [16]. To perform this evaluation, 1 mg/mL of the test material was mixed with 2 mL of 0.1 mM DPPH solution prepared in ethanol. The mixture was incubated in the dark for 30 minutes. The absorbance was measured at a wavelength of 517 nm. Ethanol was used as the blank, while a mixture of 2 mL of DPPH solution and 1 mL ethanol served as the control. Ascorbic acid (1 mg/mL) was used as the standard for comparison. The percentage scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (1).$$

2.2.6. Characterization of silver nanoparticles

The characterization of the synthesized silver nanoparticles involved Fourier Transform Infrared (FTIR) spectroscopy using a Bruker Alpha FTIR-ATR instrument, which recorded spectra within a wavenumber range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} , with duplicate measurements to ensure reliability and reproducibility. X-ray Diffraction (XRD) analysis was conducted using Cu-K α radiation over a 2θ range of 20-80° on a Rigaku Miniflex 600 operating at 45 kV and 40 mA to investigate the crystalline structure. The surface morphology of the sample was examined using a VEGA3 TESCAN scanning electron microscope (TESCAN, Czech Republic). The micrographs were acquired using a secondary electron (SE) detector at an accelerating voltage of 20.0 kV, a working distance of 15.0 mm, and a magnification of 1.65×10^3 , with a 20 μm scale bar. Additionally, Transmission Electron Microscopy (TEM) analysis was carried out using a Tecnai 200 kV instrument at SAIF-AIIMS, New Delhi, with samples prepared on carbon-coated copper grids to examine the morphology and size of the nanoparticles.

3. Results and discussion

The results of the green synthesis of silver nanoparticles using *Tridax procumbens* extracts consistently implicate polyphenolic and flavonoid constituents as the principal reducing and capping agents [3],[23]. As shown in Table 1, qualitative phytochemical screening, reports flavonoids, phenolics, tannins, saponins, and alkaloids in aqueous and organic extracts — a phytochemical mix capable of donating electrons to Ag^+ and stabilizing nascent Ag^0 through coordination and hydrogen bonding [21,22,24–26]. The phytochemical screening of *T. procumbens* root extract (Table 1) revealed abundant flavonoids, phenolic compounds, tannins, saponins, alkaloids, glycosides, and terpenoids, a profile broadly consistent with, but not identical to reports on leaf and

Table 1: Phytochemical screening results of *Tridax procumbens* extract

Phytochemical class	Presence in <i>Tridax procumbens</i> extract
Flavonoids	+++
Phenolic compounds (Total phenolics)	+++
Tannins	++
Saponins	++
Alkaloids	+
Glycosides	++
Steroids/Terpenoids	++

+ = weakly present;
++ = moderately present;
+++ = strongly present

aerial part extracts used for AgNP synthesis. Studies on *T. procumbens* leaves [10,11,14,18] generally highlight high levels of polyphenols and flavonoids as key contributors to Ag^+ reduction and nanoparticle stabilization, while the root extract in this study additionally shows a notable presence of tannins and saponins, which may contribute to metal-binding and capping efficiency.

The SEM images revealed predominantly spherical nanoparticles with relatively uniform size distribution, confirming the morphological consistency achieved through plant-mediated synthesis, as shown in Figure 1.

The SEM micrograph reveals irregularly shaped AgNPs assembled into dense, heterogeneous agglomerates with rough surfaces. This moderately agglomerated morphology could decrease the accessible surface area and restricts exposure of reactive surface sites, thereby influencing nanoparticle dispersibility and

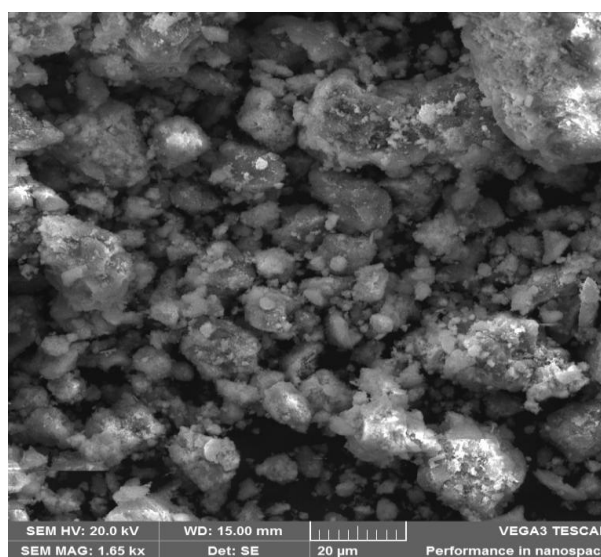


Figure 1: SEM image of *Tridax Procumbens* mediated AgNPs

surface reactivity similar to submissions of [7],[16]. Particle uniformity is critical because variations in size and shape can significantly influence biological activity, toxicity, and aggregation behavior. The small particle size may contribute to a larger surface area available for interactions with free radicals, thereby enhancing the antioxidant effect, as suggested in [16].

The EDX spectrum in Figure 2, of the synthesized nanoparticles shows predominantly silver (89.2 wt%). The presence of carbon (8.3 wt%) and oxygen (2.3 wt%) likely reflects organic phytochemicals from *Tridax procumbens* adsorbed on the supported by nanoparticle surface as reducing and capping agents [21]. The trace copper signal (0.2 wt%) probably originated from the sample holder or grid, while iron was negligible.

In the XRD spectrum shown in Figure 3, the absence of secondary diffraction peaks indicates that the synthesized material is predominantly phase-pure crystalline silver, in which no crystalline by-products or impurity phases can be detected.

The XRD pattern confirms the crystalline nature of the synthesized material. Weak reflections near 38°, 44°, 64°, and 77° are consistent with face-centred cubic silver, can be indexed to the (111), (200), (220), and (311) planes of face-centred cubic metallic silver, respectively, consistent with JCPDS/PDF No. 04-0783 while the dominant peak around 28° indicates silver oxide phase due to oxidation which aligns to [24].

The FTIR spectrum of the *Tridax procumbens*-mediated AgNPs (Figure 4) displays well-defined absorption bands

The absorption band appearing in the 3200–3600 cm⁻¹ range, which is primarily attributable to the stretching of the O–H bonds in phenolic and alcoholic groups, with a possible contribution from N–H stretching. The bands around 2850–2950 cm⁻¹ correspond to aliphatic C–H stretching, whilst the absorptions around 1600–1650 cm⁻¹ can be attributed to carbonyl, amide or aromatic C=C vibrations. The signals appearing in the 1350–1450 cm⁻¹ range originate from C–H bending and phenolic O–H deformation, whilst the intense bands in the 1000–1200 cm⁻¹ range indicate C–O and C–O–C stretching in alcohols, phenolic compounds, glycosides and related phytochemicals.

These functional groups confirm the retention of *T. procumbens* phytochemicals on the AgNP surface, where they function as reducing and capping agents. Phenolic and flavonoid-associated hydroxyl groups also provide electron- or hydrogen-donating sites that contribute to the observed DPPH radical-scavenging activity as corroborated by [16].

The TEM image shown in Figure 5 confirms that predominantly quasi-spherical AgNPs mediated by *Tridax procumbens* were formed, with a particle size of approximately 5–20 nm and minimal agglomeration. Their small size increases the availability of reactive surface sites and contact with phytochemical co-formulants, which supports the observed antioxidant effect. The study showed that the nanoparticles are predominantly spherical, which is consistent with the

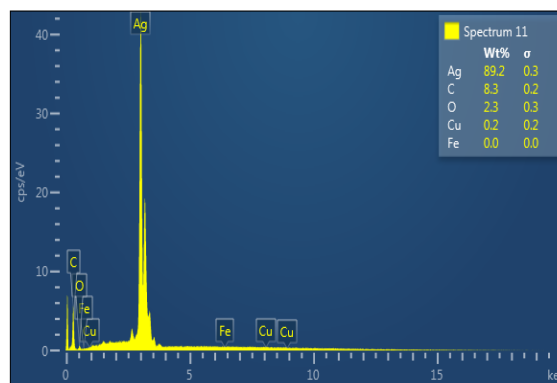


Figure 2: EDX spectrum of *Tridax procumbens* mediated AgNPs

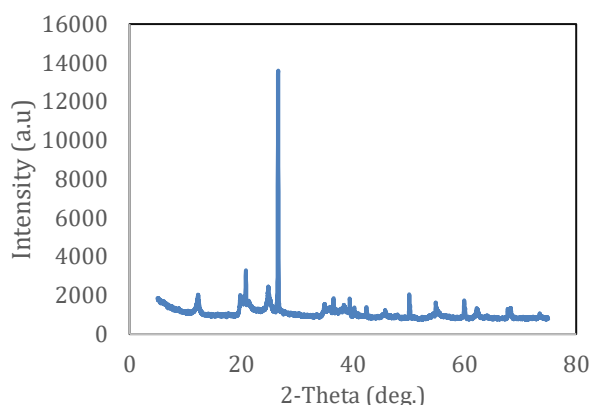


Figure 3: XRD spectrum of *Tridax procumbens* mediated AgNPs

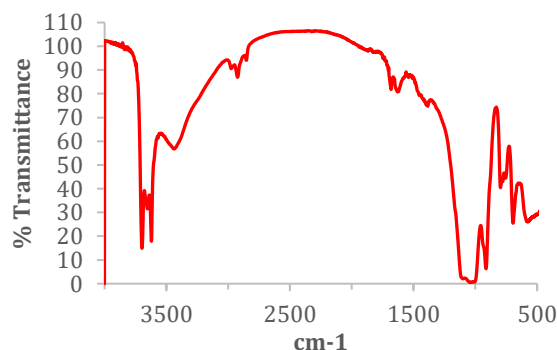


Figure 4: FTIR spectrum of *Tridax procumbens* mediated AgNPs

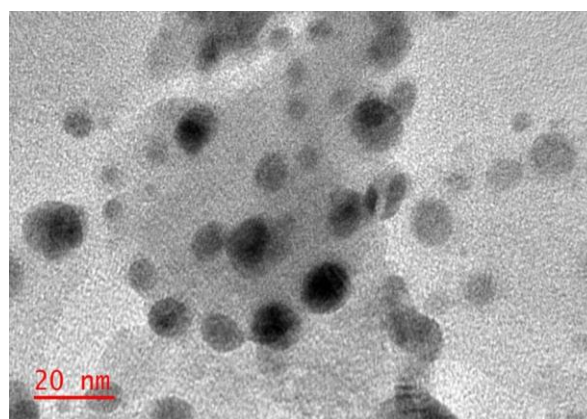


Figure 5: TEM image of *Tridax procumbens* mediated AgNPs

SEM results, and exhibit a relatively narrow size distribution, suggesting a relatively uniform particle size distribution formed during synthesis, a finding also supported by the studies [24,28,29].

Furthermore, TEM analysis provides visual evidence of minimal aggregation, reinforcing the role of biomolecules in *T. procumbens* extract as natural capping and stabilizing agents [7,30–32]. These biomolecules likely provide both steric and electrostatic stabilization, preventing particle agglomeration, which is crucial for maintaining consistent biological activity and maximizing surface-area-to-volume ratio which may contribute to antioxidant activity. In addition, the TEM analysis corroborates the deductions from SEM, XRD, and FTIR analyses, confirming that the green synthesis method using *T. procumbens* produced spherical, uniform, and relatively stable AgNPs [8,24,27,32–34]. These structural attributes may contribute to the nanoparticles' functional potential in biomedical applications.

Tables 2 and 3 show the concentration-dependent DPPH radical-scavenging ability of AgNPs and half maximal inhibitory concentration (IC_{50}) values, respectively.

DPPH radical-scavenging activity increased with rising concentration in all samples tested. AgNPs produced by *Tridax procumbens* resulted in a higher percentage of inhibition at the corresponding concentrations than the crude root extract, suggesting improved radical scavenging performance following the formation of nanoparticles. Ascorbic acid exhibited the highest activity across the entire concentration range. The IC_{50} values further reinforced this trend: ascorbic acid exhibited the lowest IC_{50} value, followed by the AgNPs, whilst the crude extract did not achieve 50 percent inhibition within the concentration range tested. These results demonstrate that the synthesized AgNPs exhibited measurable, concentration-dependent antioxidant activity, although their activity remained lower than that of the reference antioxidant.

Table 2: Concentration-dependent DPPH radical-scavenging activity of *Tridax procumbens* root extract, synthesized AgNPs, and ascorbic acid

Conc. ($\mu\text{g/mL}$)	<i>T. procumbens</i> root extract (%)	<i>T. procumbens</i> AgNPs (%)	Ascorbic acid (%)
25	18.4 $\pm$ 0.8	28.6 $\pm$ 1.1	42.7 $\pm$ 0.9
50	22.5 $\pm$ 1.0	34.9 $\pm$ 1.2	47.8 $\pm$ 1.0
100	29.8 $\pm$ 1.1	43.6 $\pm$ 1.3	61.1 $\pm$ 1.2
150	37.5 $\pm$ 1.3	53.5 $\pm$ 1.4	74.2 $\pm$ 1.1
200	46.2 $\pm$ 1.2	61.8 $\pm$ 1.5	85.9 $\pm$ 1.0

Table 3: IC_{50} values of *Tridax procumbens* root extract, synthesized AgNPs, and ascorbic acid in the DPPH assay

Sample	IC_{50}
Ascorbic acid	58.3 $\mu\text{g/mL}$
<i>T. procumbens</i> -mediated AgNPs	132.3 $\mu\text{g/mL}$
Crude root extract	>200 $\mu\text{g/mL}$

4. Conclusion

This study establishes that *Tridax procumbens* root extract is a viable biogenic medium for the synthesis and surface functionalization of AgNPs. The combined spectroscopic, structural, elemental, and microscopic evidence confirms phytochemical-mediated silver-ion reduction, biomolecular capping, crystalline phase formation, and the production of predominantly quasi-spherical nanostructures. The nanoparticles displayed greater DPPH radical-scavenging activity than the crude extract, although their activity remained below that of ascorbic acid, indicating moderate antioxidant performance. The results broaden the currently relatively limited scope of application of *T. procumbens* roots in nanomaterial synthesis and provide a basis for further structure–activity investigations.

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