

Green synthesis of gold nanoparticles using *Telfairia occidentalis* leaf extract and evaluation of their antioxidant activity for possible industrial applications

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Abstract

An investigation was conducted on the green synthesis of gold nanoparticles (AuNPs) using aqueous *Telfairia occidentalis* leaf extract as both the reducing and stabilizing agent. Following preliminary phytochemical analysis, AuNPs were synthesized by reacting aqueous tetrachloroauric acid trihydrate with the extract under controlled conditions and characterized using several analytical techniques. The UV-visible spectrum exhibited a surface plasmon resonance (SPR) band at 547.92 nm, and Fourier-transform infrared (FTIR) spectroscopy revealed hydroxyl and amide functional groups, supporting stabilization by polyphenols and proteins. High-resolution transmission electron microscopy (HRTEM) and selected-area electron diffraction (SAED) supported a quasi-spherical morphology and high crystallinity, with a mean diameter of 25.89 ± 7.53 nm, a size range of 10.81–48.31 nm, and a median diameter of 25.26 nm ($n = 108$), as determined using ImageJ; energy-dispersive X-ray spectroscopy (EDS) confirmed the elemental composition and predominance of gold. Antioxidant capacity evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), and lipid peroxidation inhibition assays was concentration dependent. At $100 \mu\text{g/mL}$, AuNPs gave $68.20 \pm 0.20\%$ DPPH inhibition versus $91.66 \pm 0.26\%$ for ascorbic acid, $75.81 \pm 0.18\%$ FRAP versus $95.88 \pm 0.38\%$, and $65.90 \pm 0.40\%$ lipid-peroxidation inhibition versus $89.25 \pm 0.40\%$. The findings indicate promising in vitro antioxidant activity, although safety, stability, and application-specific performance require further validation before biomedical or industrial use.

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1. Introduction

Gold nanoparticles (AuNPs) have drawn substantial interest lately because of their remarkable physical and chemical properties, including high surface-to-volume ratio, optical response stability, and chemical inertness [1, 2]. These characteristics

have made AuNPs attractive for several applications, including biomedicine, food, and environmental cleanup. One of the most significant applications of AuNPs is their use as antioxidant agents for preserving bioactive compounds and protecting them from oxidative degradation in industrial and biological systems [3, 4]. Gold nanoparticles synthesized using plant phytochemicals as reducing and stabilizing agents, are increasingly investigated as sustainable alternatives to conventional chemical routes [5]. A report by Ref. [6] suggests that these biogenic

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nanoparticles can exhibit enhanced biocompatibility and low intrinsic cytotoxicity in preliminary safety assessments. This method avoids the use of toxic chemicals, reduces the generation of toxic waste, and leverages the components of bioactive plants to improve the properties of the nanoparticles [7, 8].

Bio-synthesized nanoparticles have been widely investigated in current literature, with numerous studies proving the participation of plant biomolecules in the synthesis and capping of nanoparticles [9]. According to Ref. [10], flavonoids, phenolic acids, and tannins from medicinal plants play the role of reduction and stabilization agents in the synthesis of metal-based nanoparticles, resulting in higher antioxidant properties. Similarly, Ref. [11] found that the use of plant extracts improves the biocompatibility of AuNPs, which in turn reduces cytotoxicity and their potential for application in biomedicine. The importance of phytochemicals in the synthesis of nanoparticles was emphasized not only for their ability to reduce metal ions but also for their role in incorporating bioactive properties that improve their antioxidant and antimicrobial properties.

The antioxidant properties of AuNPs have been extensively explored, and it has been revealed in the literature that they possess free radical scavenging properties and the ability to inhibit lipid peroxidation [4]. AuNPs synthesized utilizing the *Moringa oleifera* extract had strong antioxidant properties comparable to commercial antioxidants such as ascorbic acid, according to Ref. [12]. In another study by Ref. [13], it was reported that gold nanoparticles combated oxidative stress in biological systems through neutralization of reactive oxygen species (ROS). It has been established that high electron density and catalytic potential of AuNPs are responsible for the removal of reactive radical species and prevent free-radical-induced deterioration of biomolecules [3, 14, 15]. In addition, incorporating plant biomolecules during synthesis significantly improves the properties, stability, and bioactivity of the nanoparticles such that they become viable for application in food preservation, nutraceuticals, and pharmaceuticals [16–19]. The antioxidant properties of AuNPs are significantly dependent on their physical and chemical properties, which include particle diameter, morphology, and surface functionalization [20–22]. Ref. [23] also highlighted that smaller AuNPs with optimal surface-to-volume ratios exhibit stronger radical scavenging ability than larger particles [24, 25].

The above reports highlight the importance of optimizing the synthesis process for the manufacture of nanoparticles possessing optimal properties for a particular application [26, 27]. The current study investigates a sustainable green approach for synthesizing environmentally friendly antioxidant agents. *Telfairia occidentalis* and *Piliostigma thonningii* were initially selected for screening due to their local availability, abundance, and recognized ethnomedicinal relevance. A preliminary phytochemical evaluation was conducted to identify the extract with superior reducing and capping capacity. Based on its higher phenolic yield, *Telfairia occidentalis* leaf extract was chosen for the green synthesis of gold nanoparticles (). Unlike conventional physicochemical routes that rely on synthetic additives for morphology control [28], this plant-mediated approach achieves dual functionalization, wherein endogenous

phytochemicals simultaneously serve as bio-reducing and stabilizing agents.

The present study reports the preparation of AuNPs using the leaf extract of *Telfairia occidentalis* and their characterization using UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), selected area electron diffraction (SAED), high-resolution transmission electron microscopy (HRTEM), and energy-dispersive X-ray spectroscopy (EDS) to analyze their structural and chemical properties. In addition, the antioxidant property of the synthesized nanoparticles was also assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), and inhibition of lipid peroxidation assays. Statistical analysis was used to compare the effectiveness of AuNPs with that of conventional antioxidants such as ascorbic acid.

2. Materials and methods

2.1. Materials

Tetrachloroauric(III) acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was obtained from Molychem India LLP, India, and used as the gold precursor. A stock solution was prepared by dissolving 1.0 g of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ in distilled water and making the volume up to 100 cm^3 , giving a concentration of 25.39 mM. The stock solution was subsequently diluted to obtain a 1 mM working solution, from which 60 cm^3 was used for the optimized synthesis. All reagents used were analytical grade. Aqueous solutions were made using distilled water. The glassware used for the experiment was new and cleaned by soaking them in nitric acid, followed by washing with distilled water to remove all contaminants. They were completely dried prior to usage.

2.2. Preparation of plant extract

Young leaves of *Telfairia occidentalis* and *Piliostigma thonningii* were collected from Batavovogi, Bida Local Government Area of Niger State, Nigeria. The leaves were washed with deionized water to remove dirt and other surface contaminants. The rinsed leaves were dried at ambient temperature for 7 days and pulverized using an agate porcelain pestle and mortar. A known weight (30 g) of each powdered sample was transferred into a 500.0 cm^3 flask containing 300.0 cm^3 of distilled water. The solution was heated at 70 °C for 120 min. The extract was allowed to cool to room temperature and was filtered through Whatman No. 1 filter paper. The filtrate was stored in an amber bottle and kept in the refrigerator at 4 °C.

2.3. Qualitative and quantitative phytochemical screening of extract

2.3.1. Quantitative phytochemical screening

2.3.2. Determination of total phenols

The total phenolic content was determined using the Folin-Ciocalteu method as described by Ref. [29]. Concentrations ranging from 0.2–1.0 mg/cm^3 of gallic acid and methanol extract of the leaf samples were prepared in methanol. Thereafter, 4.5 cm^3 of distilled water was added to 0.5 cm^3 of the extract and mixed with 0.5 cm^3 of a ten-fold diluted Folin-Ciocalteu

reagent. 5.0 cm³ of 7.5 % sodium carbonate was added to the tubes and another 2.0 cm³ of distilled water was also added. The mixture was allowed to stand for 90 min at room temperature and the absorbance was read at 765 nm. Standard gallic acid was used to prepare the calibration curve.

2.3.3. Determination of total flavonoids

The total flavonoid content of the methanolic leaf extract of the samples was determined according to the method described by Ref. [29]. About 0.5 cm³ of the extract was mixed with 1.5 cm³ of methanol, 0.1 cm³ of 1 M sodium acetate and 2.8 cm³ of distilled water, and left to stand for 30 min. The absorbance of the reaction mixture was measured at 415 nm using a double-beam Shimadzu UV-visible 1800 spectrophotometer. Standard quercetin was used to prepare the calibration curve.

2.3.4. Determination of total tannins

For each sample, 0.2 g was weighed into three 50.0 cm³ beakers, then to each, 20.0 cm³ of the 50 % methanol was added and covered with paraffin film and placed in a water bath at about 80 °C for 1 h, the mixture was shaken to ensure consistency. Afterwards, the mixtures were filtered using Whatman No. 1 filter paper into 100.0 cm³ volumetric flasks. 20.0 cm³ of water, 2.5 cm³ Folin-Denis reagent and 10.0 cm³ of 17 % Na₂CO₃ were added to each, mixed with water and allowed to stand for 20 min. The tubes were cooled to room temperature and the absorbance was measured at 760 nm using double-beam Shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve [30].

2.3.5. Determination of total alkaloids

The total alkaloid content of the crude extract was determined using the method described by Ref. [31]. 0.5 g of each sample extract was weighed and dissolved in 5.0 cm³ of mixture of 96 % ethanol and 20 % H₂SO₄ (1:1) and then filtered. 1 cm³ of the filtrate was then added to a test tube containing 5 cm³ of 60 % H₂SO₄ and allowed to stand for 5 minutes. Thereafter, 5.0 cm³ of 0.5 % formaldehyde was added and allowed to stand at room temperature for 3 hours. The absorbance was read at a wavelength of 565 nm. Vincristine extinction coefficient (E₂₉₆, ethanol (ETOH)= 15136M⁻¹cm⁻¹) was used as the reference alkaloid.

2.3.6. Determination of total saponins

The saponin content of the sample crude extracts was determined according to the method described by Ref. [32]. 0.5 g of each sample was weighed and dissolved in 20.0 cm³ of 1 M HCl and heated in a water bath at 80 °C for 4 h. The reaction mixture was cooled and filtered. 50.0 cm³ of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5.0 cm³ of acetone-ethanol (1:1), 6.0 cm³ of ferrous sulphate and 2.0 cm³ of concentrated sulphuric acid were added and allowed to stand for 10 min. The absorbance was taken at 490 nm. Standard saponin was used to prepare the calibration curve.

2.4. Green synthesis of gold nanoparticles

The synthesis of AuNPs was carried out according to Ref. [33] with slight modifications. Briefly, 60 cm³ of a 1 mM aqueous solution of tetrachloroauric(III) acid trihydrate (HAuCl₄·3H₂O) was transferred into a 250 cm³ beaker and maintained on a hot plate with a magnetic stirrer at 120 rpm until boiling began, after which the heat was turned off. Subsequently, 6.0 cm³ of aqueous *Telfairia occidentalis* leaf extract was added to the precursor solution. The precursor solution and plant extract were mixed at a 10:1 (v/v) ratio, giving a final reaction volume of 66 cm³. The reaction was conducted at the natural, unadjusted pH of the reaction mixture. Following the addition of the extract, the mixture was stirred for 15 min, during which a visible colour change from light brown to ruby red was observed, indicating the formation of AuNPs. The solution was centrifuged at 10,000 rpm for 15 min, the pellet was washed using deionized water, freeze-dried and then stored in the refrigerator at 4 °C for further analysis.

2.5. Characterization of gold nanoparticles

UV-visible spectroscopy was used to assess the optical properties of the sample using an Agilent 8453 spectrophotometer operating between 200–800 nm. A 2.0 cm³ sample in a quartz cuvette was tested against distilled-deionized water to determine the maximum absorption wavelength ($\lambda_{\max}$), which gives information about the electronic structure of the sample. For microstructural characterization, HRTEM and SAED were performed with a Zeiss Auriga instrument at 200 kV. The gold nanoparticles were dispersed in ethanol, ultrasonicated, and deposited on copper grids. FTIR spectroscopy further characterized the chemical properties by identifying surface functional groups. A mixture of the sample and potassium bromide was pressed into pellets and analyzed in the 400–4000 cm⁻¹ range utilizing a Bruker TENSOR27 spectrophotometer.

2.6. In vitro antioxidant assays

2.6.1. DPPH radical scavenging assay

To determine the antioxidant activity of the AuNPs, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed according to the method of Ref. [34]. Stock solutions (1000 µg/cm³) of gold nanoparticles and ascorbic acid were prepared by dissolving 0.01 g of each substance individually in 10 cm³ of methanol. From these stocks, serial dilutions were made to obtain working concentrations of 6.25, 12.50, 25, 50 and 100 µg/cm³. Subsequently, 1 cm³ of each concentration was mixed with 2 cm³ of 0.004 % DPPH solution. The resulting mixtures were incubated in the dark at 25 °C for 30 min. Absorbance was then measured at 517 nm against a blank using a double-beam Shimadzu UV-1800 spectrophotometer. The percentage antioxidant activity was calculated using Eq. (1):

$$\% \text{ Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100, \quad (1)$$

where A_{blank} is the blank absorbance and A_{sample} is the absorbance of the AuNPs or ascorbic acid.

2.6.2. Ferric reducing power assay

The ferric reducing power of the biosynthesized gold nanoparticles was determined according to the method of Ref. [34], with slight modifications. Stock solutions ($1000 \mu\text{g}/\text{cm}^3$) of ascorbic acid and gold nanoparticles were diluted with methanol to yield working concentrations of 6.25, 12.50, 25, 50 and $100 \mu\text{g}/\text{cm}^3$. A 1 cm^3 aliquot of each preparation was mixed with 1 cm^3 of 0.2 M sodium phosphate buffer and 1 cm^3 of 1 % potassium hexacyanoferrate(III). After a 20-minute incubation at 50°C , the reaction was stopped by adding 1 mL of 10 % trichloroacetic acid (TCA), followed by centrifugation for 10 min at room temperature. Next, 1 cm^3 of the supernatant was combined with 1 cm^3 of deionized water and 0.2 cm^3 of 0.1 % ferric chloride. A blank was prepared using deionized water in place of the extracts. Absorbance values were measured at 700 nm.

2.6.3. Inhibition of lipid peroxidation

The inhibitory effect of the extracts against lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS) assay, as described by Ref. [35] with slight modifications. Briefly, 0.5 cm^3 of 10 % (w/v) fresh egg yolk homogenate prepared in 1.15 % KCl buffer (pH 7.4) was added to 0.1 cm^3 of extract or ascorbic acid at varying concentrations (6.25, 12.50, 25, 50 and $100 \mu\text{g}/\text{cm}^3$) and made up to 1 cm^3 with deionized water. 0.05 cm^3 of freshly prepared 0.07 M FeSO_4 solution was added to the mixtures to induce lipid peroxidation and incubated at 37°C for 30 min. To the resultant solutions, 1.5 cm^3 of 20 % (v/v) acetic acid (pH 3.5) and 1.5 cm^3 of 0.8 % (w/v) thiobarbituric acid (TBA) in 1.1% (w/v) sodium dodecyl sulphate solution were added. The resulting mixture was incubated at 95°C for 1 h and allowed to cool. Afterwards, 5 cm^3 of 1-butanol was added to each reaction mixture, centrifuged for 10 min and the top organic layer absorbance was measured at 532 nm against a reagent blank. The inhibitory percentage of lipid peroxidation was estimated using Eq. (2):

$$\% \text{ Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100, \quad (2)$$

where A-blank is the absorbance of the control reaction containing all reagents except the test sample (replaced with deionized water) and A-sample is the absorbance of the AuNPs or ascorbic acid standard.

3. Results and discussion

3.1. Phytochemical screening of plant leaves

3.1.1. Qualitative analysis

The phytochemical screening results presented in Table 1 indicate the presence and relative intensity of different classes of secondary metabolites in *Telfairia occidentalis* and *Piliostigma thonningii*. *Telfairia occidentalis* showed a strong relative presence of phenolic and flavonoid compounds (+++), together with moderate tannin content (++), while *Piliostigma thonningii* exhibited a strong relative presence of saponins

(+++), and varying levels of other phytochemical classes. Phenolic compounds, flavonoids, and tannins contain functional groups that have been associated with the reduction of metal ions and stabilization or capping of nanoparticles during green synthesis. Thus, the phytochemical profile of *Telfairia occidentalis* suggests the possible involvement of these metabolites in the reduction and stabilization of the synthesized AuNPs. The observed phytochemical profile provides preliminary evidence for the potential contribution of plant-derived metabolites to AuNP formation and stabilization.

3.1.2. Quantitative analysis

As shown in Table 2, *Telfairia occidentalis* exhibited a significantly higher total phenolic content compared to *Piliostigma thonningii*. Although both plants possess valuable phytochemical profiles, *Telfairia occidentalis* was selected as the optimal bio-reducing and stabilizing agent for gold nanoparticle synthesis due to its superior phenolic yield. Plant phenolics act as primary electron donors responsible for reducing Au^{3+} to Au^0 and capping the resulting nanostructures [33, 36]. Furthermore, capping the gold nanoparticles with these phenolic antioxidants enhances their overall biological and antioxidant capacity. These differences highlight the different abilities of each sample to function as a reducing, capping, and stabilizing agent in the synthesis of nanoparticles [37, 38]. The phenolic content in *Telfairia occidentalis* ($490.02 \pm 1.18 \text{ mg}/100 \text{ g}$) was found to be higher than that of *Piliostigma thonningii* ($196.11 \pm 1.56 \text{ mg}/100 \text{ g}$). Phenols are known to perform a reducing role in the biosynthesis of AuNPs by donating electrons [39].

This indicates that *Telfairia occidentalis* has high reducing abilities, which make it more effective in the reduction process of nanoparticles [40, 41]. Also, *Telfairia occidentalis* has a higher amount of flavonoids ($272.02 \pm 1.25 \text{ mg}/100 \text{ g}$) than *Piliostigma thonningii* ($6.17 \pm 0.30 \text{ mg}/100 \text{ g}$). Flavonoids are important in reducing gold ions as well as the stabilization of gold nanoparticles through the formation of strong bonds with their surfaces [42–44]. The high flavonoid content in *Telfairia occidentalis* reveals its capacity to synthesize gold nanoparticles with improved stability.

Tannins, which are crucial in capping and stabilization, are present in higher amounts in *Piliostigma thonningii* ($77.38 \pm 1.21 \text{ mg}/100 \text{ g}$) than in *Telfairia occidentalis* ($69.62 \pm 0.50 \text{ mg}/100 \text{ g}$). The presence of higher amounts of tannins indicates that *Piliostigma* has the potential to create a stable shield around the gold nanoparticles, which can prevent nanoparticle aggregation and improve stability [45–47]. The saponin concentration in *Piliostigma thonningii* ($106.41 \pm 1.30 \text{ mg}/100 \text{ g}$) is also higher than that of *Telfairia occidentalis* ($32.38 \pm 0.73 \text{ mg}/100 \text{ g}$). Saponins are natural surfactants that increase the colloidal stability of nanoparticles by decreasing surface tension and inhibiting nanoparticle agglomeration [48, 49]. *Telfairia occidentalis* has a higher alkaloid content ($17.21 \pm 0.61 \text{ mg}/100 \text{ g}$) than *Piliostigma thonningii* ($6.01 \pm 0.82 \text{ mg}/100 \text{ g}$). Furthermore, alkaloids have been reported to enhance the stability of gold nanoparticles by engaging in surface binding interactions, thereby preventing secondary aggregation [50, 51].

Table 1. Qualitative phytochemical results of selected plant samples.

Sample	Phenols	Flavonoids	Tannins	Saponins	Alkaloids
<i>Telfairia occidentalis</i>	+++	+++	++	+	++
<i>Piliostigma thonningii</i>	++	+	++	+++	+

Keys: + = partially present, ++ = moderately present, and +++ = highly present.

This suggests that *Telfairia occidentalis* could facilitate the prolonged stabilization of the synthesized nanoparticles [51, 52]. *Telfairia occidentalis* is unique with its high phenol and flavonoid content, which is instrumental in reducing and primarily stabilizing gold nanoparticles [45, 53]. These findings showed *Telfairia occidentalis* as the best plant for the bio-production of gold nanoparticles, offering unique advantages as shown by its phytochemical results. Ref. [54] used extracts of plant parts for the synthesis of AuNPs.

3.2. Characterization

3.2.1. UV-visible spectroscopy of green-synthesized gold nanoparticles

The UV-visible spectrum of the biosynthesized gold nanoparticles using *Telfairia occidentalis* is presented in Figure 1. The optical absorption spectrum of the colloidal solution displayed a well-defined surface plasmon resonance (SPR) absorption band with a maximum absorption wavelength ($\lambda_{\max}$) at 547.92 nm. The emergence of this distinctive SPR peak within the 500–600 nm range supports the formation of gold nanoparticles, arising from the collective oscillation of free conduction band electrons interacting with the incident electromagnetic field [55]. The broadness and specific position of the peak at 547.92 nm indicate the synthesis of polydisperse, quasi-spherical nanoparticles with moderately larger size dimensions as smaller, highly monodisperse spherical AuNPs generally resonate closer to 520 nm [56]. The relatively broad base of the SPR band also suggests a wide size distribution or slight variation in nanoparticle morphology within the aqueous medium [4]. This is similar to the results of Refs. [57, 58], who reported an SPR of 548 nm for gold nanoparticles synthesized using *Suaeda japonica* leaf extract and *Salvia sclarea* L respectively.

This reduction and stabilization result from the rich profile of biomolecules such as polyphenols, flavonoids, and proteins present within the *T. occidentalis* leaf extract. Recent studies in Refs. [59, 60] report that *T. occidentalis* aqueous extracts contain robust functional groups (including phenols, alcohols, and carboxylic acids) capable of driving the rapid biogenic reduction of metal ions into stable zero-valent nanomaterials while simultaneously providing a capping matrix that prevents critical particle agglomeration.

3.2.2. FTIR of green-synthesized gold nanoparticles

FTIR spectra of *Telfairia occidentalis* extract and gold nanoparticles in Figure 2 reveals variation in the intensity and location of peaks, which certified chemical differences and changes along with interaction processes going on during gold

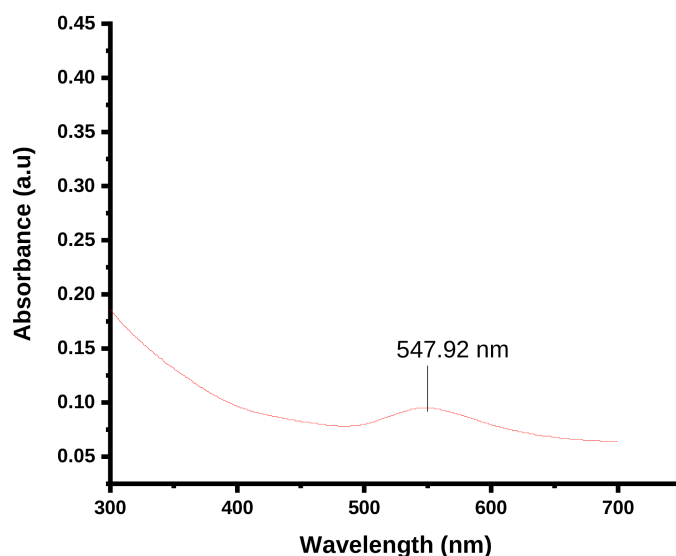


Figure 1. UV-visible spectrum of green-synthesized gold nanoparticles.

nanoparticles synthesis. The FTIR spectrum of gold nanoparticles (Figure 2a) exhibited noticeable peak shifts and intensity changes, indicating interactions between Au^{3+} ions and the plant biomolecules. The broad band at 3328 cm^{-1} (O–H/N–H stretching), together with peaks at 2904 , 2820 , 1492 , and 1216 cm^{-1} , confirmed the involvement of hydroxyl, amine, aldehydic, and polyphenolic groups in nanoparticle reduction and stabilization [61–63]. The band at 824 cm^{-1} corresponded to aromatic C–H bending [64], while the appearance of a new band at 644 cm^{-1} can be attributed to out-of-plane C–H aromatic ring bending modes of the capping phyto-constituents, as well as potential metal-ligand (Au–O) coordination interactions between the oxygen-containing functional groups of the extract and the gold nanoparticle surface, providing evidence for gold nanoparticle formation [65].

The FTIR spectrum of the *Telfairia occidentalis* aqueous leaf extract (Figure 2b) showed characteristic absorption bands corresponding to biomolecules involved in nanoparticle synthesis. A broad band at 3276 cm^{-1} was assigned to O–H and N–H stretching vibrations of hydroxyl and amine groups [66], while bands at 2872 and 2752 cm^{-1} corresponded to C–H stretching of aliphatic and aldehydic groups, respectively [67]. The peaks at 1496 and 1285 cm^{-1} were attributed to amide II (N–H bending), aromatic C=C stretching, and C–O/C–N stretching [68], whereas the bands at 908 and 800 cm^{-1} indicated out-of-plane C–H bending of aromatic compounds. These functional groups confirm the presence of phenolics, proteins, aldehydes, and other phytochemicals capable of reducing and stabilizing

Table 2. Quantitative phytochemical results (mg/100 g) of selected plant samples.

Sample	Phenols	Flavonoids	Tannins	Saponins	Alkaloids
<i>Telfairia occidentalis</i>	490.02 ± 1.18	272.02 ± 1.25	69.62±0.50	32.38 ± 0.73	17.21 ± 0.61
<i>Piliostigma thonningii</i>	196.11 ± 1.56	6.17 ± 0.30	77.38±1.21	106.41 ± 1.30	6.01 ± 0.82

The values are mean ± standard error of the mean (SEM).

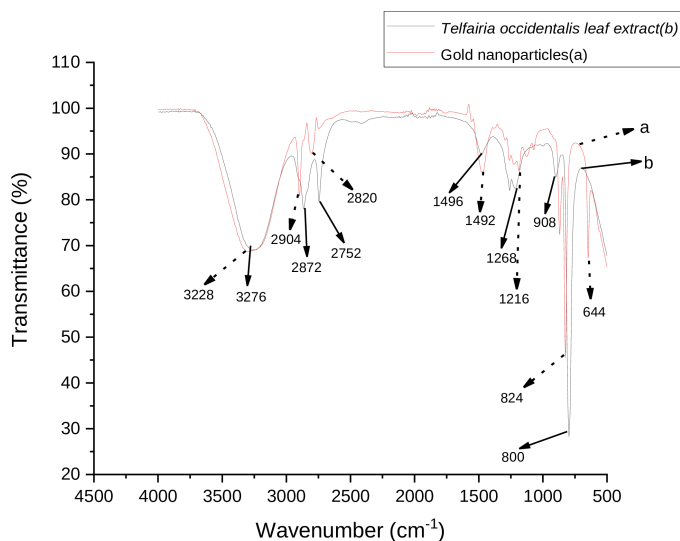


Figure 2. FTIR spectra of green-synthesized gold nanoparticles.

gold ions [69–71]. Similar FTIR profiles have been reported for *T. occidentalis* leaf extracts by Ref. [72] and [73].

The comparison of the extract and gold nanoparticle spectra revealed peak shifts, reduced band intensities, and the emergence of new absorption bands, confirming the participation of phenolic, protein, aldehydic, and amine-containing biomolecules in reducing Au^{3+} to Au^0 and stabilizing the synthesized nanoparticles [74]. The persistence of O–H, N–H, C–N, and C–O functional groups in the nanoparticle spectrum indicates that these biomolecules remained adsorbed on the nanoparticle surface acting as natural capping agents, thereby enhancing nanoparticle stability. Similar observations have been reported in Refs. [75–77].

3.2.3. HRTEM of green-synthesized gold nanoparticles

Figure 3 depicts the low- and high-magnification HRTEM images of the gold nanoparticles and the quasi-spherical shape of the nanoparticles with slight irregularities. The synthesized AuNPs exhibited a particle size range of 10.81–48.31 nm, with a mean diameter of 25.89 ± 7.53 nm and a median diameter of 25.26 nm ($n = 108$) using ImageJ software as shown in (Figure 4). The particle-size histogram showed that most of the nanoparticles were concentrated within approximately 20–30 nm, with progressively fewer particles occurring toward the lower and higher size ranges. A normal distribution fit was also applied to the measured particle size data. The Kolmogorov–Smirnov test ($p = 0.6185$) and Anderson–Darling test ($p = 0.2060$) did not indicate a statistically significant departure from normality at the 5% significance level, support-

ing the use of a normal distribution to describe the observed particle-size pattern. The relatively broad distribution may be associated with variations in nucleation and growth during the green synthesis process, which can arise from differences in the availability and concentration of phytochemicals involved in the reduction and stabilization of Au^{3+} ions [55]. There was a degree of agglomeration, with a heterogeneous population of single and clustered particles.

Even contrast across the particles suggests uniform thickness and structural homogeneity [78, 79]. High-resolution TEM images revealed clear lattice fringes, pointing to the crystallographic orientation of the gold nanoparticles [45, 80]. The measured d-spacing values are attributable to significant crystallographic planes of the face-centered cubic (FCC) crystal structure of gold [81, 82]. The d-spacing of 0.235 nm is indexed to the (111) planes, which are usually the most prominent reflections in FCC metals because of their high atomic density. Likewise, the d-spacing of 0.204 nm indexed to the (200) crystal planes, which further verifies the FCC crystalline structure [83]. These lattice fringes were continuous and showed clear atomic patterns, which indicated the presence of single-crystalline particles, while some particles showed twinning boundaries, which indicated multiple crystallographic orientations [84, 85].

The images also revealed the high crystallinity of the nanoparticles, as observed from the sharp and well-defined lattice fringes [86]. Although the individual particles are single-crystalline, others are polycrystalline, especially in the agglomerated areas [87, 88]. The boundaries of the particles are clear, and some of them have faceted surfaces, indicating controlled growth during the synthesis process [89]. The interfacial areas between the particles are also visible, which can influence the stability and interface of the particles [90]. The spherical gold nanoparticles with diameters of 12–23 nm were imaged by Ref. [91] in their research work on the effect of plasmonic resonance of gold nanoparticle doped PEDOT: PSS on the performance of CuPc/C₆₀ based organic solar cells. In the research work of Ref. [92], they described spherical particles with different sizes using different methods. The TEM analysis revealed the successful manufacture of gold nanoparticles with nanoscale dimensions. The lattice fringes and d-spaces obtained agree with the standard FCC structure of gold, which verifies the crystallinity of the synthesized gold nanoparticles [93].

These findings are consistent with Refs. [94, 95] and reflect the effectiveness of the synthesis method in manufacturing high-quality gold nanoparticles with well-ordered atomic structures and morphological features as desired. The structural homogeneity of the nanoparticles, with uniform features and controlled growth as expressed through faceted surfaces,

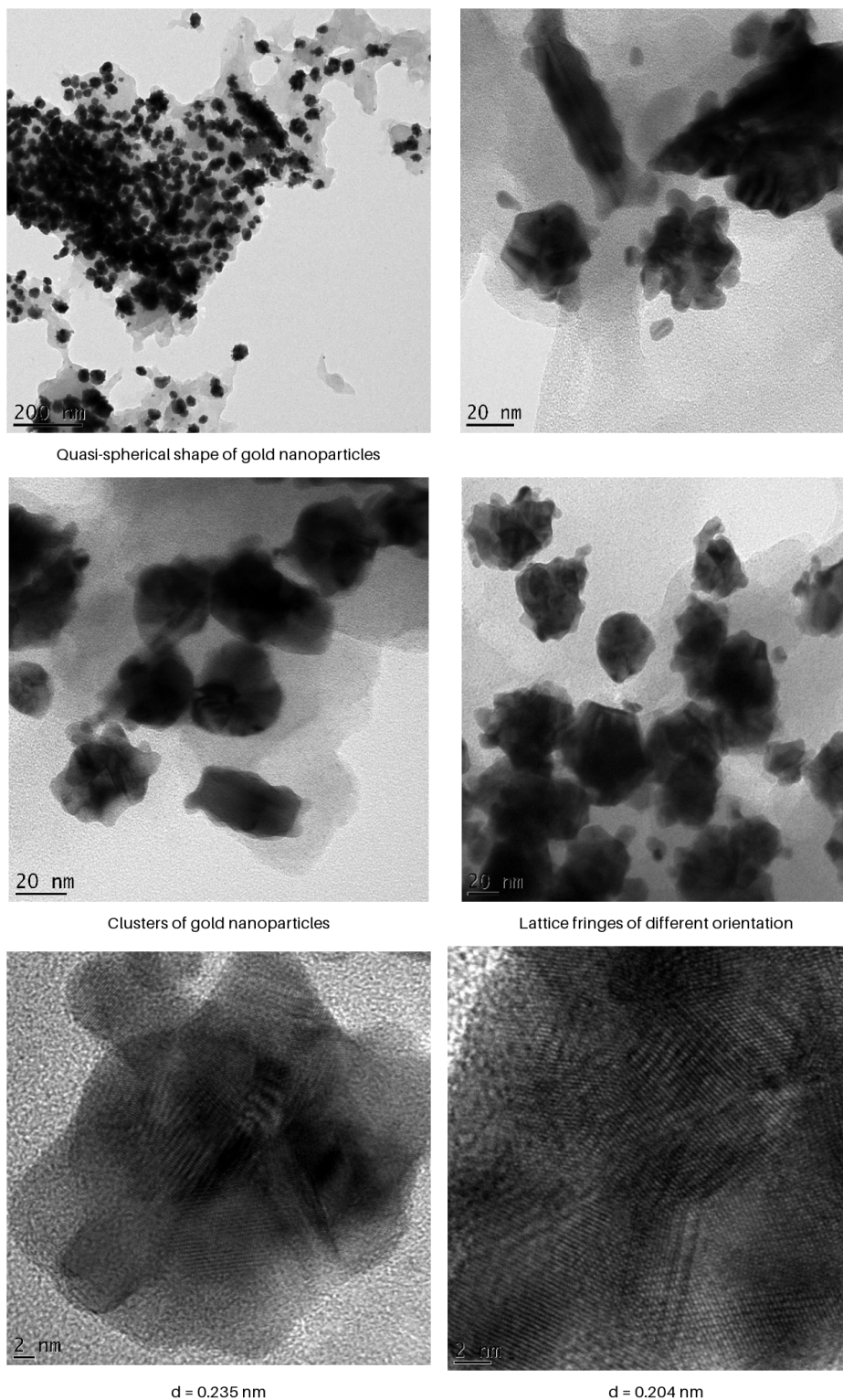


Figure 3. Low- and high-magnification HRTEM images of green-synthesized gold nanoparticles.

indicates consistent morphological characteristics of the synthesized nanoparticles [96]. Although some agglomeration was observed, the presence of single and clustered particles can enhance their stabilizing and antioxidant activity by synergistic interactions.

3.2.4. SAED of green-synthesized gold nanoparticles

The SAED pattern of the as-synthesized gold nanoparticles shows concentric rings characteristic of their crystallinity as shown in Figure 5. The pattern is consistent with the face-centered cubic (FCC) crystal structure characteristic of crys-

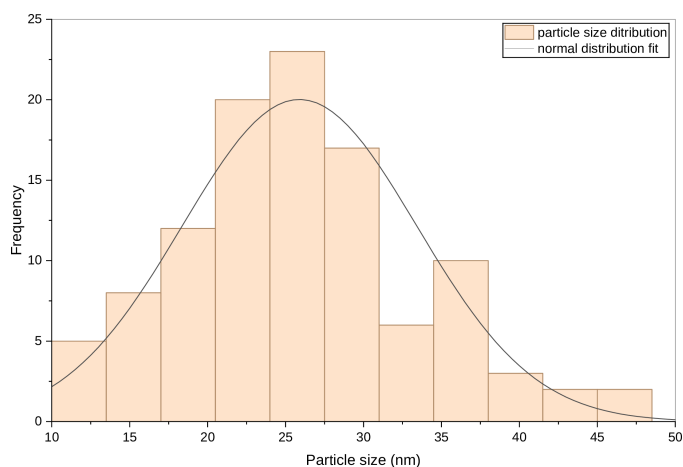


Figure 4. Particle size distribution of gold nanoparticles.

talline gold, indicating the crystalline nature and phase consistency of the synthesized AuNPs [97, 98]. The diffraction rings provided, as observed with a scale bar of 2 nm^{-1} , are indicative of specific crystalline planes. Their d-spacing values are consistent with normal FCC parameters [99]. The innermost and the strongest ring is of the (111) plane with a d-spacing of approximately $2.35 \text{ \AA}$ [100]. The plane, being the principal reflection for FCC gold, has maximum atomic density and is typically the strongest in diffraction patterns. The next ring is for the (200) plane with a d-spacing of about $2.04 \text{ \AA}$ with moderate intensity. The third and fourth rings, in the (220) and (311) planes, respectively, had d-spacing values of approximately $1.44 \text{ \AA}$ and $1.23 \text{ \AA}$ and successively lower intensities. These results confirm the expected diffraction pattern for crystalline gold nanoparticles. Similar diffraction peaks for gold nanoparticles were also noted by Ref. [101].

Sharpness and continuity of the rings in the SAED pattern also reflect the polycrystalline character of the sample, and the presence of sharp spots reflects relatively large crystallite sizes. The intensity distribution and the absence of other diffraction rings also confirm the purity of the gold nanoparticles, as there are no other phases or any other crystalline phases present. This is in accordance with the standard lattice parameters of bulk gold [97, 102].

The unique ring pattern, together with the uniformity of the concentric rings, indicates a highly ordered atomic structure [103]. The strong peak of the (111) reflection is a sign of the prevailing crystal orientation in the sample. The features evident confirm the successful synthesis of crystalline, pure gold nanoparticles with high crystallinity and uniform structural properties that may be suitable for further application [101]. This study has shown the effectiveness of the synthesis procedure in the preparation of gold nanoparticles with a well-defined FCC crystalline structure, which matches the bulk properties of gold [98]. The strong and continuous diffraction rings indicate highly defined crystallites and a polycrystalline structure, which is essential for a stable structure needed for antioxidant activity [98]. The lack of impurity phases indicates chemical purity, and their activity is only due to gold nanopar-

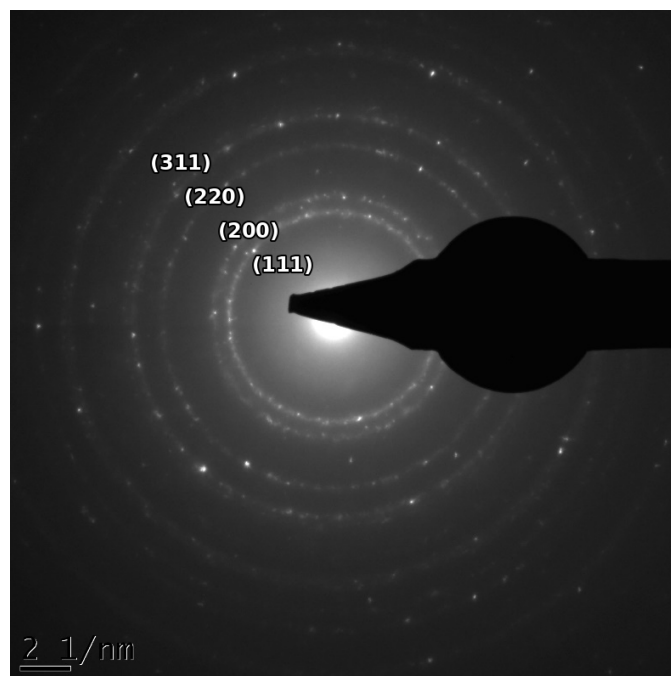


Figure 5. SAED pattern of green-synthesized gold nanoparticles.

ticles. The high atomic density of the (111) plane, high stability, and purity increase the efficiency of electron transfer and interaction with reactive oxygen species, thus increasing their radical scavenging stability. These properties make the gold nanoparticles potentially suitable for various applications subject to toxicology assessments [55].

3.2.5. EDS spectrum of green-synthesized gold nanoparticles

The EDS spectrum of the synthesized gold nanoparticles, shown in Figure 6, revealed distinct characteristic peaks corresponding to gold (Au), confirming the successful formation of AuNPs. The principal gold peaks were observed at approximately 2.12 keV ($M\alpha$), 9.71 keV ($L\alpha$), and 11.44 keV ($L\beta$) which are associated with characteristic X-ray transitions of elemental gold. The presence of these distinct Au signals provides strong evidence for the formation of metallic gold in the synthesized nanoparticles [104]. A weak signal at approximately 0.28 keV and another at approximately 8.04 keV were also observed and attributed to carbon (C) and copper (Cu) emission associated with the copper support grid used during HRTEM analysis [105]. The quantitative EDS analysis (Input full width at half maximum (FWHM) = 134 eV @ 5.9 keV Measured FWHM = 175.309 eV @ 5.9 keV Calibration: 9.98116 eV/ch , 18.6520 eV at channel 0 Accelerating voltage: 200 kV Alpha tilt: 15 degrees), further showed that Au constituted $95.86 \text{ wt } \%$ ($65.31 \text{ at } \%$) of the analysed material, while carbon accounted for $4.13 \text{ wt } \%$ ($34.68 \text{ at } \%$), as presented in Table 3.

The high weight percentage of Au is consistent with the strong and well-defined Au peaks observed in the EDS spectrum and confirms that gold was the predominant element detected in the sample. The detected carbon signal is considered

Table 3. Elemental composition of the green-synthesized AuNPs from EDS analysis.

Element	Weight (%)	Atomic (%)
C	4.13	34.68
Au	95.86	65.31
Total	99.99	99.99

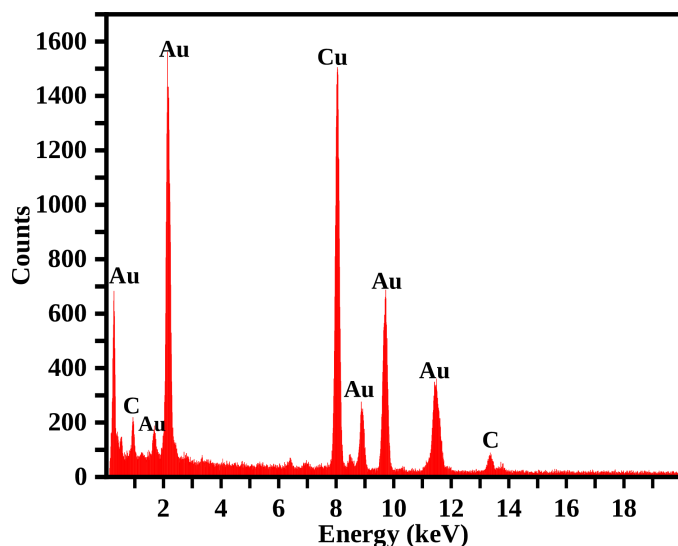


Figure 6. EDS spectrum of gold nanoparticles.

to originate primarily from the carbon support grid used during HRTEM specimen preparation and not an impurity associated with the synthesized nanoparticles. The high intensities of the characteristic Au peaks, particularly those around 2.12 and 9.71 keV, together with the relatively low background, indicate a strong Au signal. The absence of prominent signals from other metallic elements suggests that no significant metallic contaminants were detected within the analysed region [106]. The combination of the characteristic Au peaks and the quantitative elemental composition presented in Table 3 confirms the predominance of gold in the synthesized nanoparticles and supports the successful formation of the green-synthesized AuNPs [107, 108]. These findings are consistent with previous reports of characteristic EDS signals for green-synthesized gold nanoparticles [109, 110].

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

Table 4 shows that the DPPH radical scavenging activity of the synthesized gold nanoparticles was measured at varying concentrations (100–6.25 $\mu\text{g}/\text{cm}^3$) using ascorbic acid as the reference antioxidant. The gold nanoparticles exhibited concentration-dependent scavenging activity with the highest at 100 $\mu\text{g}/\text{mL}$ (68.20 ± 0.20 %) and the lowest at 6.25 $\mu\text{g}/\text{mL}$ (10.10 ± 0.25 %). However, their activity was significantly less than that of ascorbic acid at all the concentrations, which recorded the highest scavenging activity of 91.66 ± 0.26 % at 100 $\mu\text{g}/\text{cm}^3$ and 40.07 ± 0.37 % at 6.25 $\mu\text{g}/\text{cm}^3$. The IC_{50} values were 58.28 $\mu\text{g}/\text{cm}^3$ and 15.80 $\mu\text{g}/\text{cm}^3$ for AuNPs and ascorbic

acid respectively. Similarly, Ref. [111] achieved comparable findings by demonstrating that gold nanoparticles synthesized via green routes have moderate antioxidant capacity relative to conventional antioxidants like ascorbic acid. The observed activity indicates that the biosynthesized AuNPs possess antioxidant potential under the conditions investigated, as their scavenging activity confirms findings from previous studies underlining their mild but potential antioxidant activity [3].

3.3.2. Ferric reducing power

The reducing power of the gold nanoparticles prepared was measured over the concentration range 100–6.25 $\mu\text{g}/\text{cm}^3$ and compared with ascorbic acid, a known standard antioxidant (Table 5). The reducing activity of the gold nanoparticles was concentration dependent with maximum at 100 $\mu\text{g}/\text{cm}^3$ (75.81 ± 0.18 %) and minimum at 6.25 $\mu\text{g}/\text{cm}^3$ (13.94 ± 0.52 %). Conversely, ascorbic acid possessed greater reducing potential at all the concentrations ranging from 95.88 ± 0.38 % at 100 $\mu\text{g}/\text{cm}^3$ to 43.01 ± 0.52 % at 6.25 $\mu\text{g}/\text{cm}^3$. The IC_{50} values were 47.91 $\mu\text{g}/\text{cm}^3$ and 8.90 $\mu\text{g}/\text{cm}^3$ for AuNPs and ascorbic acid. Ref. [6] reported a similar finding in their studies and stated that gold nanoparticles fabricated with green procedures possess moderate ferric reducing ability compared to the usual antioxidants.

In addition, Ref. [112] emphasized that the reducing capacity of nanoparticles results from their size, surface area, and the existence of bioactive compounds from the synthesis media, which may intensify or limit their antioxidant activity. The observed differences between the gold nanoparticles and ascorbic acid align with the study by Ref. [113], which established that nanoparticles possess inherent reducing power, but their activity is typically lower than that of synthetic antioxidants due to their difference in surface chemistry. These results justify the applicability of gold nanoparticles as secondary reducing agents, beneficial in applications where the moderate antioxidant activity is desired.

3.3.3. Lipid peroxidation

Table 6 shows the protective activity of biosynthesized gold nanoparticles against lipid peroxidation assessed at a concentration of 100 to 6.25 $\mu\text{g}/\text{cm}^3$ in comparison with ascorbic acid as the standard reference.

Gold nanoparticles showed concentration-dependent activity with the highest inhibition at 100 $\mu\text{g}/\text{cm}^3$ (65.90 ± 0.40 %) and the lowest at 6.25 $\mu\text{g}/\text{cm}^3$ (5.63 ± 0.34 %). Nevertheless, ascorbic acid had significantly higher inhibitory activity throughout all concentrations, ranging from 89.25 ± 0.40 % at 100 $\mu\text{g}/\text{cm}^3$ to 34.87 ± 0.48 % at 6.25 $\mu\text{g}/\text{cm}^3$. The IC_{50} values were 63.93 $\mu\text{g}/\text{cm}^3$ and 24.51 $\mu\text{g}/\text{cm}^3$ for gold nanoparticles and ascorbic acid. This is consistent with the findings of Ref. [114] that green synthesized gold nanoparticles inhibit lipid peroxidation to a moderate level in comparison with powerful antioxidants like ascorbic acid.

Similarly, Ref. [115] noted that bioactive molecules not only cap gold nanoparticles in green synthesis but also enhance their antioxidant activity, albeit to a smaller degree than synthetic antioxidants. Differences between the gold nanoparticles

Table 4. DPPH radical scavenging activity of synthesized gold nanoparticles.

Sample	Concentration ($\mu\text{g}/\text{cm}^3$)				
	100	50	25	12.50	6.25
Gold	68.20 $\pm$ 0.20	55.86 $\pm$ 0.31	35.57 $\pm$ 0.21	23.75 $\pm$ 0.27	10.10 $\pm$ 0.25
Ascorbic acid	91.66 $\pm$ 0.26	78.09 $\pm$ 0.20	57.91 $\pm$ 0.28	45.47 $\pm$ 0.08	40.07 $\pm$ 0.37

The values are mean $\pm$ standard deviation of triplicate determinations.

Table 5. Ferric reducing activity of the synthesized gold nanoparticles.

Sample	Concentration ($\mu\text{g}/\text{cm}^3$)				
	100	50	25	12.50	6.25
Gold	75.81 $\pm$ 0.18	62.63 $\pm$ 0.14	40.49 $\pm$ 0.20	28.93 $\pm$ 0.35	13.94 $\pm$ 0.52
Ascorbic acid	95.88 $\pm$ 0.38	83.95 $\pm$ 0.27	60.96 $\pm$ 0.27	50.07 $\pm$ 0.14	43.01 $\pm$ 0.52

The values are mean $\pm$ standard deviation of triplicate determinations.

Table 6. Percentage inhibition of the synthesized gold nanoparticles on lipid peroxidation.

Sample	Concentration ($\mu\text{g}/\text{cm}^3$)				
	100	50	25	12.50	6.25
Gold	65.90 $\pm$ 0.40	52.03 $\pm$ 0.10	30.23 $\pm$ 0.33	21.08 $\pm$ 0.14	5.63 $\pm$ 0.34
Ascorbic acid	89.25 $\pm$ 0.40	73.75 $\pm$ 0.32	51.99 $\pm$ 0.08	41.33 $\pm$ 0.29	34.87 $\pm$ 0.48

The values are mean $\pm$ standard deviation of triplicate determinations.

and ascorbic acid corroborate earlier findings by Ref. [116], which indicated that although nanoparticles exhibit promising antioxidant activity, the activity is a function of the synthesis method and nature of bioactive molecules. The results indicate the potential of gold nanoparticles as inhibitors of lipid peroxidation with potential applications in scenarios where moderate oxidative stress relief is needed.

The antioxidant activity of the green-synthesized AuNPs may arise through several complementary mechanisms rather than a single pathway. The DPPH radical-scavenging activity may involve hydrogen atom transfer (HAT) and/or electron transfer (ET) from phytochemical constituents associated with the nanoparticle surface to the stable DPPH radical, thereby reducing and stabilizing the radical species [117]. The FRAP reaction primarily reflects electron-donating or reducing capacity, whereby surface-associated antioxidant molecules facilitate the reduction of Fe^{3+} to Fe^{2+} . The observed inhibition of lipid peroxidation may further indicate interference with the propagation phase of lipid oxidation through scavenging of lipid radicals and reduction or decomposition of lipid hydroperoxides [118]. The antioxidant contribution of the AuNPs results from the combined effects of the metallic gold core and the phytochemical shield formed during green synthesis [119]. Phenolic and flavonoid constituents of *Telfairia occidentalis* extract may act as hydrogen or electron donors and may remain associated with the nanoparticle surface after synthesis, thereby contributing to radical neutralization and protection against oxidative processes [120].

The high radical scavenging activity and protection against lipid oxidation observed in this study can be directly attributed to the synergistic effect between the gold metallic core and the surface capping phytoconstituents of *Telfairia occidentalis*.

These bio-functional attributes could directly translate to significant industrial applicability [121]. In the food industry, their capacity to scavenge peroxy radicals enables their application as bio-based nanostabilizers to prevent rancidity in edible oils and as active agents in food packaging films [3]. In the cosmetics industry, the synergistic combination of the metallic gold core with surface-bound phenolic hydroxyl and flavonoid groups offers potent photoprotective and free-radical scavenging properties suitable for anti-aging and topical skin formulations [122]. Furthermore, in the pharmaceutical and biomedical fields, the eco-friendly, chemical-free synthesis route yields biocompatible nanostructures with active surface functional groups (-OH, -COOH) ideal for drug conjugation and target-specific anti-inflammatory therapies [123]. The ability to combine these bio-functional properties summarizes the functional application of *T. occidentalis* synthesized gold nanoparticles as potential sustainable alternatives to synthetic additives upon further safety tests.

4. Conclusion

The green synthesis of AuNPs utilizing aqueous extracts of *Telfairia occidentalis* leaf has been successfully demonstrated under the reported laboratory conditions in this study. The phytochemical screening analysis established the presence of bioactive compounds such as phenols and flavonoids, which were responsible for reducing and stabilizing the gold nanoparticles. The characterization methods such as UV-visible spectroscopy, FTIR, HRTEM, SAED, and EDS analysis confirmed the successful production of gold nanoparticles with a quasi-spherical shape, high crystallinity, and purity. The antioxidant activity of the synthesized AuNPs was determined using DPPH

radical scavenging, ferric reducing antioxidant power, and lipid peroxidation inhibition. The findings revealed that the AuNPs possess moderate antioxidant activity, which is dose-dependent. Although they are less effective compared to ascorbic acid in reducing power and radical scavenging activity, they were found to be active and possess good antioxidant properties, indicating their potential use as additional antioxidants. These results add to the body of knowledge on green nanotechnology. Future studies should be directed at optimizing the synthesis conditions, assessing the long-term stability, biocompatibility, and toxicity of the nanoparticles.

Data availability

The data supporting the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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