

Original Article

Eco-friendly production of gold nanoparticles from *Ocimum sanctum* leaves and their application in catalytic reduction of nitro-organic compounds in industrial wastewater

Produção ecológica de nanopartículas de ouro a partir de folhas de *Ocimum sanctum* e sua aplicação na redução catalítica de compostos nitro-orgânicos em efluentes industriais

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Abstract

The growing need for sustainable and environmentally benign methods in nanotechnology has led to increased interest in plant-mediated synthesis of metal nanoparticles. This study synthesized gold nanoparticles (AuNPs) using an eco-friendly, green chemistry approach employing *Ocimum sanctum* (holy basil) leaf extract as a reducing and stabilizing agent. The bio-fabricated AuNPs were characterized using UV-Vis spectroscopy, FTIR, XRD, TEM, SEM, and elemental mapping analysis to determine their optical properties, functional groups, crystalline nature, and morphology. UV-Vis spectroscopy revealed a characteristic surface plasmon resonance (SPR) peak at approximately 536 nm, indicating the successful synthesis of AuNPs. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the nanoparticles, displaying distinct diffraction peaks corresponding to the face-centered cubic (fcc) structure of gold. Transmission electron microscopy (TEM) further illustrated the morphology of the synthesized AuNPs, revealing predominantly spherical particles with a uniform size distribution ranging from 7 to 37 nm. The synthesized nanoparticles exhibited excellent catalytic activity in reducing toxic nitro-organic compounds, such as 4-nitrophenol and 4-nitroaniline, commonly found in industrial wastewater. Kinetic studies confirmed that the reduction followed pseudo-first-order kinetics in the presence of NaBH_4 . This green synthesis approach provides a sustainable alternative to conventional chemical methods and demonstrates practical utility in environmental remediation applications. The results suggest that *Ocimum sanctum*-derived AuNPs could serve as efficient, low-cost, and eco-friendly catalysts for detoxifying industrial effluents.

Keywords: Gold nanoparticles, eco-friendly production, *Ocimum sanctum*, catalytic reduction, industrial wastewater.

Resumo

A crescente necessidade por métodos sustentáveis e ambientalmente corretos em nanotecnologia levou a um aumento do interesse na síntese de nanopartículas metálicas mediada por plantas. Neste estudo, nanopartículas de ouro (AuNPs) foram sintetizadas por meio de uma abordagem de química verde e ecologicamente correta, empregando extrato da folha de *Ocimum sanctum* (popularmente conhecido como manjerição-sagrado) como agente redutor e estabilizante. As AuNPs biofabricadas foram caracterizadas por espectroscopia UV-Vis, FTIR, XRD, TEM, SEM e análise de mapeamento elementar para determinar suas propriedades ópticas, grupos funcionais, natureza cristalina e morfologia. A espectroscopia UV-Vis revelou um pico característico de ressonância plasmônica de superfície (SPR) em aproximadamente 536 nm, confirmando a síntese bem-sucedida de AuNPs. A análise de difração de raios X (XRD) confirmou a natureza cristalina das nanopartículas, exibindo picos de difração distintos correspondentes à estrutura cúbica de face centrada (FCC) do ouro. A microscopia eletrônica de transmissão (MET) evidenciou a morfologia das AuNPs sintetizadas, revelando partículas predominantemente esféricas com distribuição uniforme de tamanho variando de 7 a 37 nm. As nanopartículas sintetizadas exibiram excelente atividade catalítica na redução de compostos nitro-orgânicos tóxicos, como 4-nitrofenol e 4-nitroanilina, comumente encontrados em efluentes industriais. Estudos cinéticos confirmaram que a redução seguiu uma cinética de pseudoprimeira ordem na presença de NaBH_4 . Essa abordagem de síntese verde não apenas fornece uma alternativa sustentável aos métodos químicos convencionais, como também demonstra utilidade prática em aplicações de remediação ambiental. Os resultados sugerem que as AuNPs derivadas de *Ocimum sanctum* podem servir como catalisadores eficientes, de baixo custo e ecologicamente corretos para a desintoxicação de efluentes industriais.

Palavras-chave: nanopartículas de ouro, produção ecologicamente correta, *Ocimum sanctum*, redução catalítica, efluentes industriais.

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Received: January 11, 2025 – Accepted: May 10, 2025

Editor: Takako Matsumura Tundisi



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1. Introduction

Metallic nanoparticles have garnered considerable interest due to their distinctive optical, magnetic, and catalytic properties. These characteristics are strongly influenced by factors such as particle size, shape, uniformity, and structural configuration (Suganthi et al., 2018; Islam et al., 2019). Among the various types of metal nanoparticles, noble metals, particularly copper, mercury, silver, platinum, and gold, have attracted significant attention in the research community (Hu et al., 2020). Gold stands out for its exceptional stability and has been widely investigated for its unique optical, chemical, and electronic properties, which make it highly applicable in fields such as pharmacology, drug delivery, cosmetics, lubrication, and catalysis (Baruah et al., 2018; Zhao and Jin, 2018). Gold nanoparticles (AuNPs), specifically, offer promising applications in cancer therapy, surface-enhanced Raman spectroscopy (SERS), and infrared radiation absorption (Aljabali et al., 2018). Various synthetic techniques encompassing chemical, physical, and biological methods have been developed to produce AuNPs with diverse sizes and morphologies (Aljabali et al., 2018; Deyev et al., 2017; Khutale and Casey, 2017). Although chemical and physical methods are commonly employed, they often require toxic reagents and non-polar solvents, which raise environmental concerns and involve expensive purification steps (Botteon et al., 2021). In response to these challenges, green synthesis approaches using plant extracts as bio-reducing agents have emerged as environmentally friendly alternatives. Phytochemicals present in plants can effectively reduce metal ions and facilitate the formation of nanoparticles with a range of functional properties (Folorunso et al., 2019).

Plant-mediated biological synthesis of nanoparticles is gaining significant attention due to its simplicity, cost-effectiveness, and environmentally benign nature. Plants possess a diverse array of phytochemicals capable of reducing metal ions from higher to zero oxidation states, making them effective agents in nanoparticle biosynthesis (Islam et al., 2019). Compared to other biological systems, plant extracts offer a more practical and scalable approach for nanoparticle production, with reduced health and environmental risks (Kumar et al., 2019). Bioactive compounds such as alkaloids, phenolic acids, polyphenols, proteins, sugars, and terpenoids found in plant extracts are believed to play essential roles in both the reduction of metal ions and stabilization of nanoparticles during synthesis (Thangamani and Bhuvaneshwari, 2019; Rastogi et al., 2018).

Ocimum sanctum Linn, commonly known as Holy Basil or Tulsi, is a medicinal plant native to Southeast Asia and widely used in Ayurvedic medicine. This short-lived perennial, which grows at elevations up to 6000 feet in the Himalayas, has been traditionally valued for its culinary, therapeutic, and industrial uses (Singh and Chaudhuri, 2018). Its rich phytochemical profile makes it a strong candidate for green nanoparticle synthesis.

Nitro-aromatic compounds (NACs) contain one or more nitro groups and are synthetic pollutants primarily originating from industrial and anthropogenic activities (Bilal et al., 2021). These compounds are released into the environment through industrial discharge, transportation, storage, and improper waste disposal, contributing to

widespread contamination of air, soil, and water ecosystems. Due to their persistence and toxicity, NACs pose serious risks to human health and the environment (Tiwari et al., 2019).

In this study, we report a green synthesis method for gold nanoparticles (AuNPs) using leaf extracts of *Ocimum sanctum* as both reducing and stabilizing agents. The process is rapid, cost-effective, reproducible, and free from toxic chemicals, aligning with sustainable nanotechnology principles. Characterization of the synthesized AuNPs was performed using UV-Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX). The biosynthesized AuNPs' catalytic activity was evaluated in reducing common nitro-organic pollutants such as nitrophenol, nitroaniline, and aminophenol compounds frequently found in industrial wastewater.

This research introduces a novel, green synthetic route for AuNPs utilizing *Ocimum sanctum*, a phytochemically rich yet underexplored plant for nanoparticle production. Unlike conventional physical and chemical methods, this biosynthetic approach eliminates hazardous reagents and high energy requirements. Moreover, the study demonstrates the dual functionality of the synthesized AuNPs as both eco-friendly nanomaterials and effective catalysts for degrading hazardous pollutants in wastewater, offering a sustainable solution for environmental remediation.

2. Material and Methods

2.1. Materials

Gold (III) chloride trihydrate was purchased from Sigma-Aldrich, South Africa. All aqueous solutions were prepared using deionized water. *Ocimum sanctum* leaves were collected from the Durban area in KwaZulu-Natal, South Africa.

2.2. Methods

2.2.1. Plant collection and extraction of leaf extract

Leaves of *Ocimum sanctum* were collected from various locations in and around Durban, KwaZulu-Natal, South Africa, by Professor H. Baijnath, a plant taxonomist at the University of KwaZulu-Natal. The collected leaves were thoroughly washed with deionized water to remove any surface impurities, then finely chopped and immersed in 200 mL of deionized water in a 500 mL conical flask. The mixture was heated for 20 minutes with continuous stirring using a magnetic stirrer. After heating, the extract was allowed to cool to room temperature and filtered through Whatman No. 1 filter paper into a sterile container for further use.

2.2.2. Green Synthesis of Gold Nanoparticles

In a 250 mL Erlenmeyer flask, 5 mL of the aqueous *Ocimum sanctum* extract was added to 45 mL of a 1 mM HAuCl_4 solution. The mixture was stirred at room temperature using a magnetic stirrer. A visible color change to ruby red was observed, indicating the formation of gold

nanoparticles. The reduction of Au^{3+} ions and nanoparticle formation were monitored at regular intervals using a UV-Vis spectrophotometer, as described by Ramakrishna et al. (2016).

2.3. Characterization of the synthesized nanoparticles

2.3.1. UV-vis spectrophotometer

The formation of gold nanoparticles was confirmed using UV-Visible spectroscopy, conducted with a Varian Cary 100 UV-Vis spectrophotometer equipped with WinUV software. Samples were placed in quartz cuvettes and scanned over a wavelength range of 200 to 800 nm. Absorbance values were recorded in absorbance units (AU).

2.3.2. Transmission Electron Microscopy (TEM)

The shape and size of the synthesized gold nanoparticles (AuNPs) were analyzed using Transmission Electron Microscopy (TEM). Samples were prepared by centrifuging the AuNP solutions at 1300 rpm for 45 minutes. The supernatant was discarded, and the resulting pellet was resuspended in distilled water. A drop of the suspension was then placed on a carbon-coated copper grid and allowed to air-dry at room temperature. TEM analysis was conducted using a JEOL JEM-1010 transmission electron microscope equipped with a MegaView III camera and iTEM UIP software (Tokyo, Japan) at the University of KwaZulu-Natal, Westville Campus (South Africa).

2.3.3. Fourier-Transform Infrared (FTIR) spectroscopy

To determine if the same biomolecules were responsible for both the production and capping of the AuNPs in the extracts, FTIR studies were conducted. Initially, the AuNP solutions were centrifuged at 1300 rpm for 45 minutes, the supernatant was discarded, and the residues were resuspended in distilled water. A drop of the material was then placed on a PerkinElmer Spectrum 100 FTIR spectrophotometer. A scan was carried out to determine the transmittance percentage across a wavenumber range of 500 to 4000 cm^{-1} . The results were analyzed using E-FTIR software to identify peak values, which were subsequently assessed (Patel et al., 2020).

2.3.4. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analysis (EDX)

A drop of the gold nanoparticle suspension was deposited onto an aluminum stub and air-dried to facilitate water evaporation. The dried sample was then examined using a ZEISS LEO 1450 Scanning Electron Microscope (conventional SEM with X-ray microanalysis) at the University of KwaZulu-Natal to visualize the morphology and confirm the presence of gold nanoparticles. To determine the elemental composition of the nanoparticles, Energy Dispersive X-ray (EDX) analysis was performed in conjunction with the SEM.

2.4 Catalytic reduction of 4-nitrophenol and 4-nitroaniline

In a standard quartz cuvette, 1 mL of ice-cold aqueous sodium borohydride (NaBH_4 , 15 mM) was mixed with 1.7 mL of 4-nitrophenol (0.2 mM). The reaction formed

4-nitrophenolate ions, changing the solution color from light yellow to yellowish-green. Subsequently, 0.3 mL of the gold nanoparticle (AuNP) solution was added to the reaction mixture at room temperature. UV-Vis absorbance spectra were recorded at 0.5-minute intervals over a wavelength range of 200 to 700 nm. The same procedure was followed for the catalytic reduction of 4-nitroaniline, following the method described by Anand et al. (2015).

3. Results and Discussion

3.1. Green synthesis of gold nanoparticles using *Ocimum sanctum* extract

The initial confirmation of gold nanoparticle (AuNP) synthesis was visually observed through a distinct color change in the reaction mixture. When a gold salt solution, typically chloroauric acid (HAuCl_4), was treated with *Ocimum sanctum* leaf extract, the solution underwent a visible transformation from pale yellow to ruby red. This color shift serves as a qualitative indicator of nanoparticle formation and is attributed to the surface plasmon resonance (SPR) phenomenon a unique optical property of metal nanoparticles.

As shown in Figure 1A, the original gold ion solution exhibits a light yellow color due to the presence of unreacted Au^{3+} ions. Upon the gradual addition of the *Ocimum sanctum* aqueous extract (rich in reducing phytochemicals like eugenol, flavonoids, and polyphenols), a reduction reaction occurs, converting Au^{3+} ions into elemental gold (Au^0). This nucleation and subsequent growth of gold atoms lead to the formation of nanoscale particles. Figure 1B captures the final stage of the reaction, where the solution attains a characteristic ruby red color.

(MubarakAli et al., 2011). The plant extract contains phytochemicals such as flavonoids, phenolics, and terpenoids, which act as natural reducing agents. These biomolecules reduce Au^{3+} ions to Au^0 atoms when introduced into the gold salt solution. As these atoms begin to nucleate and grow into nanoparticles, a distinct ruby red color emerges in the colloidal solution (Karthick et al., 2014; Inbakandan et al., 2010; Kumari and Meena, 2020). This red coloration arises due to the Surface Plasmon Resonance (SPR) effect, a unique optical phenomenon exhibited by metal nanoparticles, particularly noble metals like gold. SPR occurs when the conduction band electrons on the surface

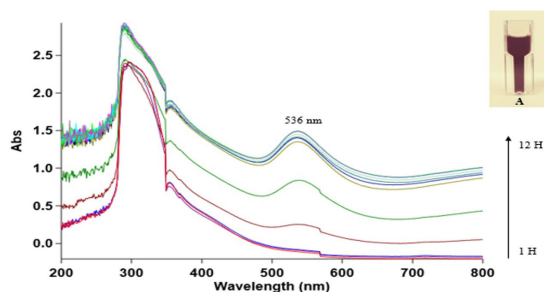


Figure 1. UV-vis scan of (A) Gold nanoparticles, (B) HAuCl_4 , (C) *O. sanctum* leaf extract at different times.

of the gold nanoparticles collectively oscillate in resonance with the electromagnetic field of incident light (typically in the visible range) (Balasubramanian et al., 2020).

3.2. UV-visible spectroscopy

The successful synthesis of gold nanoparticles (AuNPs) using *Ocimum sanctum* leaf extract was confirmed by UV-Visible spectroscopic analysis, a widely employed technique for monitoring metallic nanoparticles' optical properties and stability. A prominent Surface Plasmon Resonance (SPR) band was observed in the final reaction mixture, providing strong evidence of nanoparticle formation. As shown in Figure 1, the UV-Vis absorbance spectra display a distinct and well-defined SPR peak centered at 536 nm, which is a characteristic absorption maximum for colloidal gold nanoparticles. This SPR band arises due to the coherent oscillation of free conduction electrons on the surface of the nanoparticles when excited by incident light. The interaction between the electromagnetic field of the light and the conduction electrons leads to a collective oscillation, resulting in a sharp absorption peak in the visible region of the spectrum (Li et al., 2018; Rance et al., 2008). For instance, Valsalam et al. (2019) reported the formation of gold nanoparticles using *Musa acuminata* collar flower extract, where the SPR band was observed at 540 nm. This slight red shift compared to the 536 nm peak in the current study can be attributed to differences in particle size, shape, and capping agents derived from the plant extracts. The phytochemical profile of *Musa acuminata* likely differs from that of *O. sanctum*, influencing both the reduction kinetics and nanoparticle stabilization, which in turn affects the optical absorption characteristics.

Metallic nanoparticles exhibit unique optical properties, most notably Surface Plasmon Resonance (SPR), which is attributed to the collective excitation of conduction band electrons on the nanoparticle surface when exposed to incident light (Manju et al., 2016). These electrons oscillate in resonance with the electromagnetic field, and the specific vibrational mode is determined by the nanoparticle's size, shape, and surrounding environment. SPR is a distinctive feature of noble metal nanoparticles, such as gold, that

significantly enhances electromagnetic fields at their surface, thereby amplifying optical phenomena including light scattering and absorption (Suriyakala et al., 2022).

In the current study, the progress of gold nanoparticle (AuNP) formation was monitored via UV-Vis spectroscopy, revealing a dynamic change in absorbance intensity over time. Within the first 4 hours of the reaction, a shift in absorbance intensity to 0.838 was observed, indicating initial nanoparticle nucleation. As the reaction progressed, this intensity increased steadily, reaching a maximum absorbance of 1.495 at 536 nm after 12 hours (see Figure 1). The peak at 536 nm is a characteristic SPR band for gold nanoparticles, confirming their formation and stabilization in the colloidal suspension.

Control experiments were conducted individually using chloroauric acid (HAuCl₄) and *O. sanctum* leaf extract. In both cases, no absorbance peaks were detected between 510 and 540 nm, which are the typical SPR wavelengths for AuNPs. This absence of SPR signatures indicates that no gold nanoparticles were formed in the absence of the complete reaction system. Furthermore, no color change was observed in the chloroauric acid solution without the addition of *O. sanctum* extract, suggesting that the phytochemicals present in the leaf extract act as effective reducing agents, essential for the conversion of Au³⁺ to Au⁰ and the subsequent nanoparticle formation.

3.3. Fourier Transform Infrared (FTIR) spectroscopy analysis

Fourier-transform infrared (FT-IR) spectroscopy was employed to identify the functional groups involved in the reduction of gold ions (Au³⁺) and the subsequent capping and stabilization of the synthesized gold nanoparticles (AuNPs). The FT-IR spectrum of the AuNPs (Figure 2) displayed several distinct absorption bands that indicate the presence of organic functional groups associated with bioactive compounds from the *Ocimum sanctum* leaf extract.

Key absorption peaks were observed at 1017, 1229, 1511, 1634, and 2917 cm⁻¹, each corresponding to specific molecular vibrations: The high-frequency band at 2917 cm⁻¹ lies within the alkane (C-H) stretching region and is indicative of flavonoids, which are known to possess -CH₂ and -CH₃ groups.

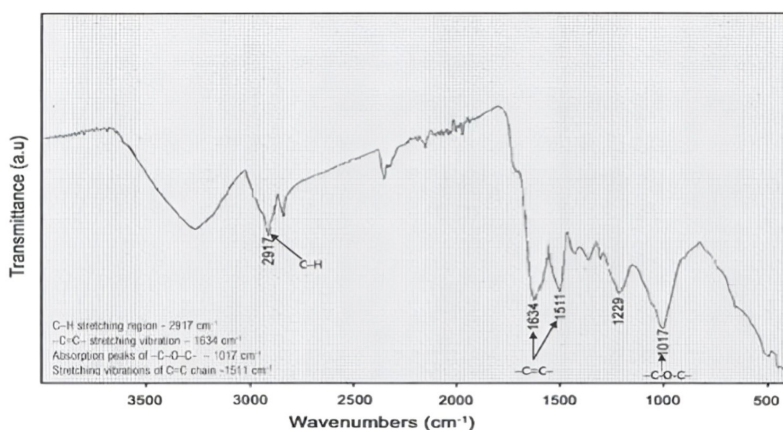


Figure 2. FTIR spectrum of gold nanoparticles obtained from *O. sanctum* leaf extract.

The strong absorption peak at 1634 cm^{-1} is characteristic of C=C stretching vibrations, suggesting the presence of alkene functional groups, commonly found in phytochemicals like flavonoids and terpenoids. The 1511 cm^{-1} band also corresponds to C=C stretching, reinforcing the presence of conjugated alkene chains or aromatic systems.

The absorption band at 1229 cm^{-1} can be attributed to C–O stretching vibrations, potentially from phenolic or alcoholic groups. The 1017 cm^{-1} band is associated with C–O–C ether linkages, which may originate from glycosidic bonds or other oxygen-containing compounds in the extract.

These vibrational bands are strongly linked to water-soluble phytochemicals such as flavonoids, terpenoids, polyphenols, and other aromatic compounds present in *O. sanctum* (Pattanayak et al., 2010). Their presence in the FT-IR spectra of the synthesized AuNPs suggests that these biomolecules act as reducing agents facilitating the transformation of Au^{3+} to Au^0 and function as natural capping agents, binding to the nanoparticle surface and preventing aggregation. This dual role enhances the stability and dispersion of the nanoparticles in the aqueous medium, demonstrating the effectiveness of the green synthesis route using *O. sanctum* leaves.

3.4. TEM analysis of morphology and size distribution of biogenic AuNPs

The size, shape, and surface morphology of the gold nanoparticles (AuNPs) synthesized using *Ocimum sanctum* leaf extract were characterized using Transmission Electron Microscopy (TEM). The TEM micrographs (Figure 3) reveal that most biosynthesized AuNPs are spherical in shape, with particle sizes ranging from 7 to 37 nm. This size distribution is consistent with SPR characteristics observed in UV-Vis analysis and confirms the successful formation of nano-sized particles. In addition to spherical shapes, a subset of the larger nanoparticles exhibited diverse morphologies, including triangles, hexagons, and circular platelets, typical structural variants observed

in gold nanoparticle synthesis (Suriyakala et al., 2022). These non-spherical forms may result from anisotropic growth processes during the later stages of nanoparticle formation. The synthesis of AuNPs was facilitated by hydrogen bonding and electrostatic interactions between the bioactive compounds in *O. sanctum* leaf extract and the surface of the forming Au^0 particles. These interactions play a crucial role in reducing Au^{3+} ions and capping the nanoparticles, stabilizing the particles and preventing uncontrolled agglomeration.

However, the presence of larger and irregularly shaped nanoparticles suggests partial aggregation of smaller particles, which is common in biosynthetic routes due to less controlled nucleation kinetics. This size variation can be attributed to the multi-phase nature of biologically mediated nanoparticle synthesis, where nucleation and growth occur asynchronously. Furthermore, Rajasekharreddy et al. (2010) noted that limited availability of reducing agents in plant extracts often favors the formation of spherical nanoparticles, which are energetically more stable under mild reaction conditions. Thus, the present study's predominance of spherical AuNPs aligns with this plant-mediated nanoparticle formation model.

3.5. SEM analysis of surface morphology

Scanning Electron Microscopy (SEM) was employed to investigate further the surface morphology, shape, and particle distribution of the gold nanoparticles (AuNPs) synthesized using *Ocimum sanctum* leaf extract. As shown in Figure 4, the SEM micrographs confirm the spherical morphology of most AuNPs, consistent with observations from TEM analysis. The particles appeared within the nanoscale range, with some aggregates measuring around 200 nm, indicating clustering of individual nanoparticles. The SEM images revealed a dense distribution of nanosized crystalline particles, typical of biologically synthesized metal nanoparticles. Notably, many particles appeared to be surrounded by a faint halo, a phenomenon often attributed

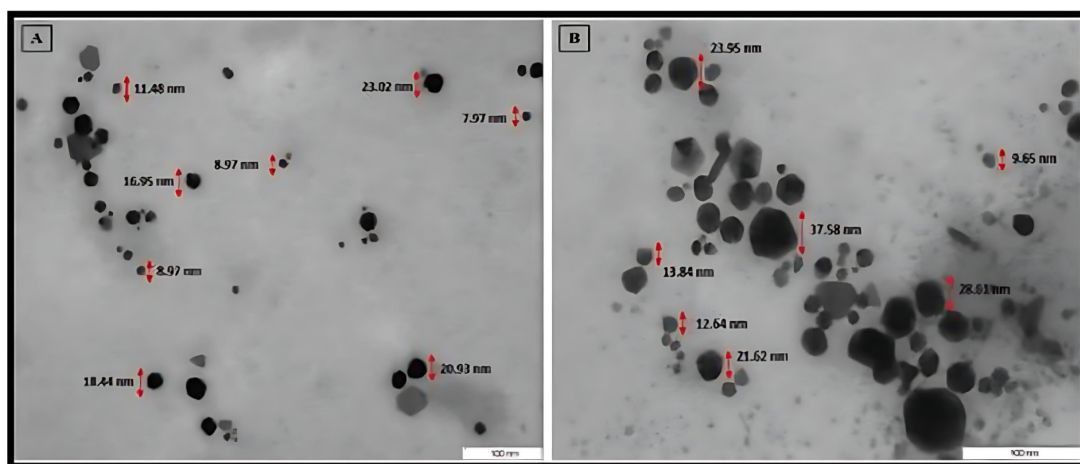


Figure 3. TEM images showing gold nanoparticles synthesized from *Ocimum sanctum* leaf extract at 100 nm magnification. (A) Gold nanoparticles showing particle sizes ranging from 7.97 to 23.02 nm. (B) Gold nanoparticles showing larger particle sizes ranging from 9.65 to 37.58 nm. Red arrows indicate measured nanoparticle diameters.

to the presence of organic capping layers from the plant extract. These halos suggest that phytochemicals from the *O. sanctum* extract facilitated reduction and formed a protective coating around the nanoparticles, aiding in their stabilization and preventing immediate oxidation or agglomeration. The occurrence of particle aggregation in the micrographs can be explained by the high surface energy of nanoparticles, which often leads to clustering, especially in dried SEM samples. This behavior is consistent with findings reported by Nagalingam et al. (2018), who observed similar aggregated spherical gold nanoparticles synthesized using *Alternanthera bettzickiana* extracts.

The SEM analysis supports the conclusions drawn from UV-Vis, FT-IR, and TEM data, further validating the successful biosynthesis and morphological stability of AuNPs using *Ocimum sanctum* as a green reducing and capping agent.

3.6. Energy-dispersive X-ray spectra

The elemental composition and confirmation of metallic gold in the biosynthesized nanoparticles were

assessed using Energy-Dispersive X-ray Spectroscopy (EDX). The EDX spectrum (Figure 5) exhibited a distinct optical absorption peak at approximately 2.2 keV, which is characteristic of metallic gold (Au^0) and is consistent with previous reports by Elavazhagan and Arunachalam (2011). This strong signal confirms the successful reduction of chlorauric acid (HAuCl_4) to elemental gold, supporting the conclusions drawn from UV-Vis, FT-IR, and TEM analyses. The spectrum displays ionization energy (keV) on the x-axis and detected X-ray counts on the y-axis, with clear gold peaks detected at multiple points, indicating homogenous distribution of gold across the sample. Strong and weak gold signals were recorded at six distinct spots, reinforcing the uniformity and consistency of gold nanoparticle formation. In addition to gold, several other elemental signals were observed, providing insight into the biomolecular capping agents derived from the *Ocimum sanctum* leaf extract. The quantitative EDX analysis revealed high weight percentages of oxygen (28%) and silicon (28%), which are common in plant-derived samples and may be associated with

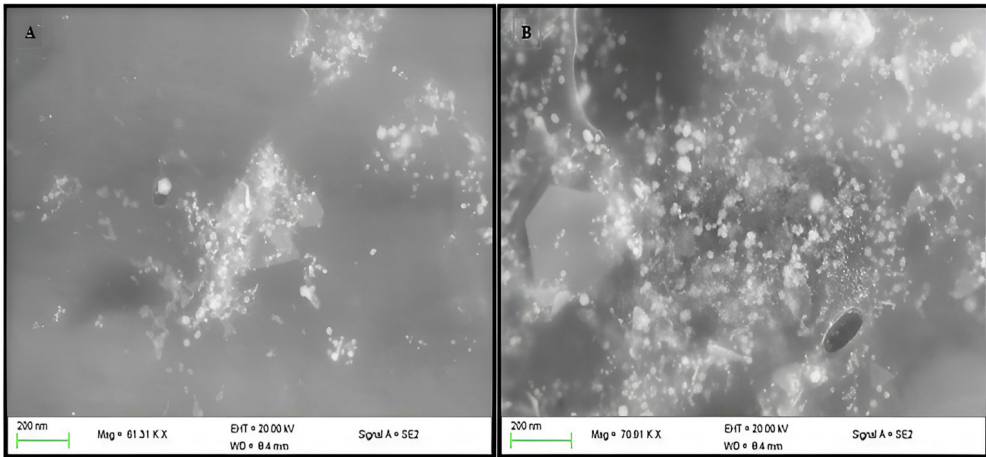


Figure 4. SEM micrographs of gold nanoparticles synthesized from the reaction of chlorauric acid and *Ocimum sanctum* leaf extract. Scale bar = 200 nm. (A) shows relatively dispersed spherical gold nanoparticles, while (B) highlights aggregated nanoparticle clusters, indicating varying nanoparticle assembly degrees.

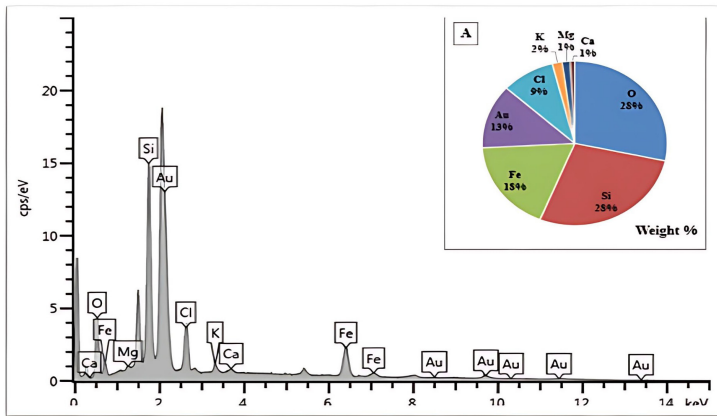


Figure 5. EDX spectroscopy with SEM analysis of gold nanoparticle. Insert A shows the quantitative analysis (weight %) of the elements found in the sample.

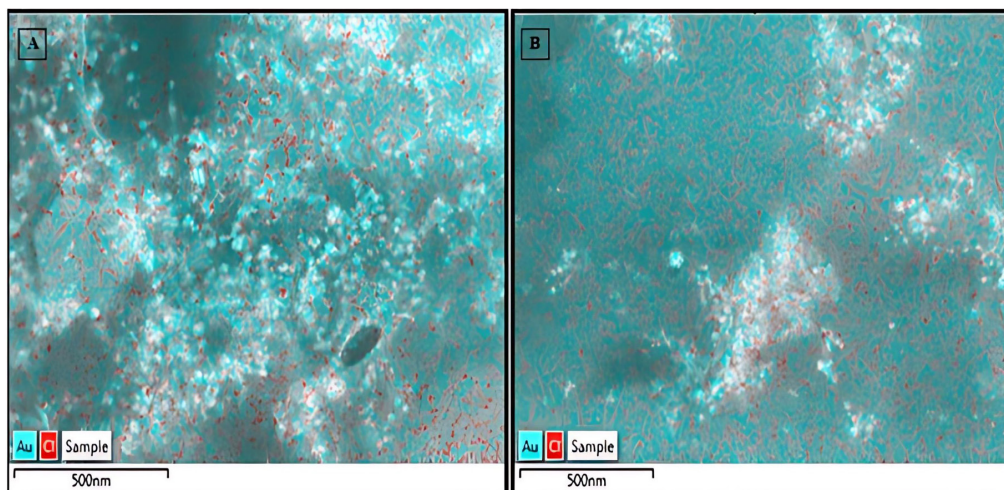


Figure 6. Distribution of gold (Au), chlorine (Cl), and gold conjugates in the gold nanoparticle solution observed at 500× magnification. (A) indicates regions rich in elemental gold, while (B) highlights areas corresponding to gold conjugates associated with chlorine.

organic compounds and residual plant matter. A notable iron content (18%) was also detected, aligning with iron's known biological role in plant tissues.

Trace elements including calcium (1%), potassium (2%), and magnesium (1%) were also present, consistent with the mineral composition of *O. sanctum* leaves as reported by Pattanayak et al. (2010). The presence of chlorine (9%) likely originates from residual chloroauric acid, whereas gold was detected at 13% by weight, confirming its substantial incorporation into the synthesized nanomaterial. The EDX data validate the biosynthetic origin and elemental purity of the AuNPs, highlighting the role of naturally occurring phytochemicals and minerals in nanoparticle formation, capping, and stabilization.

3.7. Elemental mapping analysis of the gold nanoparticles

To further analyze the spatial distribution of elements within the biosynthesized gold nanoparticle sample, elemental mapping was performed in conjunction with scanning electron microscopy (SEM). The results, presented in Figure 6, provide visual evidence of the localization and interaction of gold (Au) and chlorine (Cl) across different regions of the nanoparticle matrix. In the elemental maps, blue areas represent gold (Au), red areas denote chlorine (Cl), and white areas correspond to gold conjugates, possibly indicating gold nanoparticles capped or stabilized by plant biomolecules. In Figure 6A, the gold conjugates appear to be evenly dispersed throughout the sample, surrounded by a well-defined presence of both gold and chlorine. This uniform distribution suggests effective capping and stabilization by phytochemicals present in the *Ocimum sanctum* extract, supporting the hypothesis that bioactive molecules facilitate the reduction of Au³⁺ and contribute to nanoparticle surface modification and spatial organization. Conversely, Figure 6B illustrates a sample region with a less dense distribution of gold conjugates. While gold and chlorine remain uniformly scattered, the reduced density of gold conjugates in this area indicates

localized variations in nanoparticle concentration. This non-uniform distribution may arise from natural heterogeneity in the plant extract, differences in nucleation kinetics, or local fluctuations in the availability of reducing and capping agents during synthesis.

These observations support the conclusion that plant-derived biomolecules play a crucial role in nanoparticle formation and stabilization, highlighting the inherent variability of biosynthetic methods. The presence of chlorine alongside gold further confirms residual traces of the gold precursor (HAuCl₄). In contrast, the distribution of gold conjugates reinforces the green synthesis process's effectiveness but not absolute uniformity.

3.8. Catalytic activity of AuNPs in the reduction of 4-Nitrophenol and 4-Nitroaniline

The catalytic reduction of nitro-organic compounds, such as 4-nitrophenol and 4-nitroaniline, is a widely studied reaction due to the environmental significance of nitro-aromatic pollutants. In this process, sodium borohydride (NaBH₄) serves as a reducing agent, while gold nanoparticles (AuNPs) act as catalysts for the reduction of the nitro group (NO₂) to an amine group (NH₂) (Seo et al., 2017; Anand et al., 2015). The reaction proceeds with the formation of a yellowish 4-nitrophenolate anion, as NaBH₄ donates electrons to the nitro group, converting it to an amine form which exhibit a strong absorbance peak at 400 nm. In the absence of AuNPs, this peak remained unchanged for several hours, demonstrating that the reaction is kinetically hindered without a catalyst. However, in the presence of the biosynthesized AuNPs, a gradual decline in absorbance at 400 nm was observed (Figure 7A), and the solution became colorless within 20 minutes, signifying the complete reduction of 4-NP to 4-aminophenol (Jayabal and Ramaraj, 2014).

A similar catalytic effect was observed in reducing 4-nitroaniline (4-NA). Initially, 4-NA showed an absorbance peak at 380 nm. After the addition of AuNPs and NaBH₄, the absorbance steadily decreased, and the solution turned

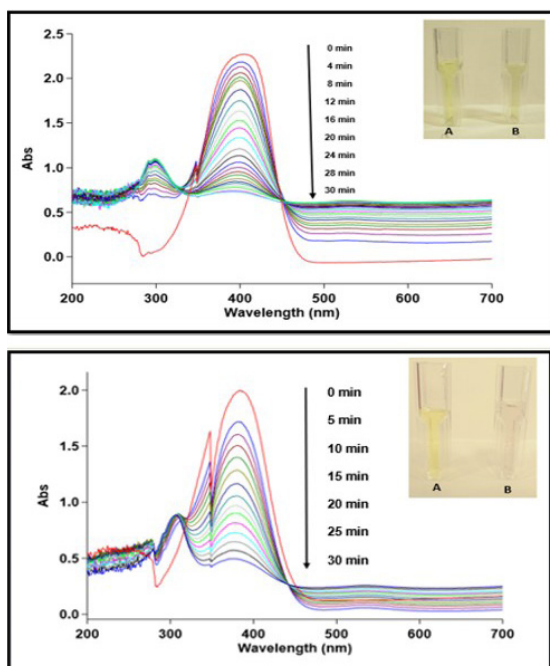


Figure 7. A time trend graph of AuNPs catalysing the reduction of (A) 4-nitrophenol and (B) 4-nitroaniline.

colorless within 20 minutes (Figure 7B), indicating the formation of 4-phenylenediamine (Rajamanikandan et al., 2023). In contrast, control reactions without AuNPs showed no significant change in absorbance or color, even after extended periods. The enhanced catalytic activity of the AuNPs can be attributed to their high surface-to-volume ratio, which provides more active sites for substrate interaction, and their rapid surface adsorption kinetics (Singh et al., 2018; Kumari and Meena, 2020). The AuNPs act as electron relay centers, facilitating the transfer of electrons from BH_4^- to the nitro group, effectively reducing the nitroaromatic compounds under mild, room-temperature conditions (Pradhan et al., 2002).

The successful catalytic reduction of nitro-organic compounds using biogenic gold nanoparticles has significant practical implications, especially in treating industrial wastewater containing nitro-aromatic pollutants. Nitro-aromatic compounds, such as 4-nitrophenol and 4-nitroaniline, are commonly found in wastewater from industries like pharmaceuticals, dyes, and chemicals (Sharma et al., 2016). These pollutants are toxic, non-biodegradable, and pose serious risks to aquatic ecosystems and human health if not properly treated. Using gold nanoparticles synthesized from *Ocimum sanctum* leaves offers an eco-friendly, sustainable, and cost-effective solution to address this challenge. The synthesis method is green, avoiding the use of harmful chemicals, and the gold nanoparticles themselves are highly effective at reducing nitro-organic compounds, making them promising candidates for use in wastewater treatment processes (Bhushan et al., 2024).

By applying these nanoparticles in wastewater treatment systems, it may be possible to reduce the concentration of harmful nitro-aromatic pollutants to safer levels. The

catalytic properties of AuNPs can be utilized in flow-through or batch reactors to treat industrial effluents continuously. Additionally, these nanoparticles' relatively low-cost and scalable production and their high catalytic efficiency make them an attractive alternative to traditional treatment methods, which often rely on harsh chemicals, high energy consumption, or complex technologies. Furthermore, the stability and reusability of gold nanoparticles in catalytic processes suggest that they could be employed in regenerative treatment systems, where they can be reused multiple times without significant activity loss. This further enhances the sustainability of the process, reducing operational costs and environmental impact (Rodrigues et al., 2019).

These findings highlight the potential of green-synthesized AuNPs as efficient catalysts for deleting nitro-organic pollutants in industrial wastewater. Their ability to function in aqueous media without harsh reaction conditions further underscores their suitability for eco-friendly wastewater treatment applications.

4. Conclusion

In conclusion, the use of *Ocimum sanctum* leaves for the eco-friendly synthesis of gold nanoparticles (AuNPs) presents a sustainable, cost-effective alternative to conventional nanoparticle production methods. The green synthesis approach harnesses the plant's natural reducing and stabilizing properties, offering a non-toxic, environmentally friendly pathway for nanoparticle fabrication. The synthesized AuNPs demonstrated remarkable catalytic activity in reducing nitro-organic pollutants, such as 4-nitrophenol and 4-nitroaniline, under mild conditions, showcasing their potential for environmental remediation. These findings suggest that *O. sanctum*-derived gold nanoparticles could significantly address pollution in industrial wastewater, providing a viable solution for treating hazardous effluents. With their high efficiency, biocompatibility, and eco-friendly synthesis, AuNPs represent a promising avenue for green nanotechnology in environmental applications.

Future research efforts should focus on optimizing the synthesis parameters, exploring the scalability of the process, and investigating the long-term stability and reusability of the nanoparticles in real-world industrial scenarios. Further studies on the broader application of green nanomaterials could offer novel solutions to sustainability challenges across various sectors, making significant strides toward pollution mitigation and a more sustainable industrial landscape.

Acknowledgements

The authors thank the South African National Research Foundation (NRF) for its support.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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