

Green Synthesis of Gold Nanoparticles (AuNPs) Using Butterfly Pea Flower (*Clitoria ternatea*) Extract: Effect of Reaction Time on Particle Characteristics

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ABSTRACT

This study investigated the green synthesis of gold nanoparticles (AuNPs) using *Clitoria ternatea* (butterfly pea) flower extract as a natural reducing and stabilizing agent and evaluated the physicochemical properties of the synthesized nanoparticles. Nanoparticle formation was initially confirmed by a gradual color change in the reaction mixture from pale yellow to ruby red. UV-Visible spectroscopy revealed a characteristic Surface Plasmon Resonance (SPR) absorption band centered at approximately 545.5 nm, with the highest absorbance recorded after 90 min, indicating the optimum reaction time for nanoparticle formation. Fourier Transform Infrared (FTIR) spectroscopy identified O-H, C-H, C=C, and C-O functional groups, confirming the involvement of phytochemical constituents in the reduction of Au³⁺ ions and stabilization of the resulting nanoparticles. Scanning Electron Microscopy (SEM) analysis demonstrated the formation of nanoscale particles with heterogeneous morphologies and slight agglomeration. Overall, the results confirm that *C. ternatea* flower extract provides an effective, sustainable, and environmentally benign route for AuNP synthesis, highlighting its potential as a plant-based bioreductant for future nanotechnology applications

INTRODUCTION

Nanotechnology has attracted considerable research interest because materials engineered at the nanoscale display unique physicochemical properties that are substantially different from those observed in their bulk forms. Owing to their dimensions, typically between 1 and 100 nm, nanomaterials exhibit increased surface area, enhanced chemical reactivity, and distinctive optical, electronic, and catalytic properties. These unique characteristics have enabled their extensive application in numerous sectors, including medicine, environmental remediation, catalysis, sensing technologies, and energy-related systems (Joudeh & Linke, 2022; Khan et al., 2022; Naga Mallikarjun Rao & Vakkalagadda, 2024). Among these materials, gold nanoparticles (AuNPs) have attracted considerable attention because of their excellent chemical stability, biocompatibility, and unique optical properties arising from Surface Plasmon Resonance (SPR), which enable their application in sensing, catalysis, imaging, and biomedical diagnostics (Bai et al., 2020; Jain et al., 2007).

Conventional methods for synthesizing gold nanoparticles commonly employ hazardous reducing agents, such as sodium borohydride, and organic solvents that may pose risks to both environmental and human health (Niznik et al., 2024). In addition, these approaches often require energy-intensive or environmentally unfavorable reaction conditions. Therefore, the development of safer, more efficient, and sustainable synthetic routes has become an important research focus (Marcelino et al., 2021).

The application of plant extracts in green synthesis has attracted significant interest as a sustainable approach to nanoparticle production, owing to the presence of naturally occurring phytochemicals that function as both reducing and capping agents. Among various plant-based materials, butterfly pea flower (*Clitoria ternatea*) is a promising candidate because it contains abundant phytochemicals, including flavonoids, anthocyanins, tannins, and other polyphenolic compounds with strong antioxidant and reducing properties (Fatimah et al., 2020; Jeyaraj et al., 2022). These compounds are capable of donating electrons for the reduction of Au^{3+} ions into metallic gold (Au^0) while simultaneously adsorbing onto the nanoparticle surface to improve colloidal stability.

Despite the increasing interest in plant-mediated nanoparticle synthesis, the influence of reaction time on the green synthesis of gold nanoparticles using *Clitoria ternatea* flower extract has not been systematically investigated. Reaction time is a critical parameter because it influences the reduction of Au^{3+} ions, nanoparticle nucleation and growth, particle size distribution, stability, and optical properties (Aji et al., 2020).

Therefore, a better understanding of its role is essential for optimizing the synthesis process and improving nanoparticle quality. This study aimed to synthesize gold nanoparticles using *C. ternatea* flower extract through a green synthesis approach and to evaluate the effect of reaction time on nanoparticle formation and characteristics. The novelty of this work lies in the systematic investigation of reaction-time-dependent AuNP formation using *C. ternatea* as a dual-function reducing and stabilizing agent. The findings are expected to

provide valuable insights into the optimization of environmentally friendly AuNP synthesis and contribute to a better understanding of plant-mediated nanoparticle formation mechanisms.

THEORETICAL REVIEW

Green synthesis has emerged as an environmentally sustainable approach for the fabrication of metallic nanoparticles, providing a greener alternative to conventional chemical methods that typically employ hazardous reducing agents and organic solvents (Santhosh et al., 2022). Among the available biological resources, plant extracts have attracted considerable attention because they are rich in naturally occurring phytochemicals, including phenolic compounds, flavonoids, and other antioxidant metabolites, which can function simultaneously as reducing and stabilizing agents. These bioactive constituents donate electrons to reduce Au^{3+} ions to elemental gold (Au^0) while concurrently adsorbing onto the nanoparticle surface, thereby preventing aggregation and improving colloidal stability. As a result, the phytochemical composition of the plant extract plays a critical role in regulating nanoparticle formation, influencing particle size, morphology, size distribution, and long-term stability (Eltahir et al., 2024).

Clitoria ternatea has attracted considerable attention because of its high content of anthocyanins, flavonoids, and polyphenolic compounds (Sangeetha et al., 2026). Previous studies have demonstrated the potential of *C. ternatea* extract in nanoparticle synthesis and other green nanotechnology applications. These bioactive compounds are capable of donating electrons during the reduction process and may simultaneously act as capping agents that prevent nanoparticle aggregation. Such characteristics make *C. ternatea* a promising biological resource for sustainable nanoparticle production (Fatimah et al., 2020; Vidana Gamage et al., 2021)

Previous investigations have demonstrated that the physicochemical characteristics of biosynthesized AuNPs are strongly influenced by several experimental parameters, including precursor concentration, extract dosage, pH, reaction temperature, and reaction duration (Ahmad et al., 2016; Sallem et al., 2026; Yazdani et al., 2021). Among these parameters, reaction time plays a particularly important role because it determines the extent of metal ion reduction and regulates the transition from nanoparticle nucleation to particle growth, ultimately influencing nanoparticle size distribution and colloidal stability (Yazdani et al., 2021). However, studies specifically investigating the effect of reaction time on the green synthesis of AuNPs using *C. ternatea* flower extract remain limited. Consequently, a systematic evaluation of reaction-time-dependent nanoparticle formation is needed to improve process optimization and enhance understanding of the synthesis mechanism.

To address this knowledge gap, the present study systematically investigates the influence of reaction time on the green synthesis of gold nanoparticles (AuNPs) using *Clitoria ternatea* flower extract as both a reducing and stabilizing agent. The findings are expected to improve the understanding of reaction kinetics during nanoparticle formation, facilitate the optimization of

sustainable AuNP synthesis, and support the broader utilization of plant-derived biomaterials in nanotechnology.

The study employed *Clitoria ternatea* flower extract as a natural reducing and stabilizing agent, chloroauric acid (HAuCl₄) as the gold precursor, and distilled water (H₂O) throughout the synthesis process. Additional reagents, including ethanol (CH₃CH₂OH) and pH-adjusting solutions (NaOH and HCl), were used when required during the synthesis process.

The equipment employed in this study consisted of standard laboratory glassware, including beakers, Erlenmeyer flasks, volumetric flasks, and pipettes, as well as a magnetic stirrer equipped with a hot plate. The synthesized gold nanoparticles were characterized using UV-Visible (UV-Vis) spectroscopy to confirm nanoparticle formation, Fourier Transform Infrared (FTIR) spectroscopy to identify the functional groups involved in the reduction and stabilization processes, and Scanning Electron Microscopy (SEM) to investigate particle morphology.

Research Design

This study employed an experimental laboratory design to synthesize gold nanoparticles (AuNPs) via a green synthesis approach using *Clitoria ternatea* flower extract. The experimental procedure consisted of three sequential stages: (1) preparation of the *Clitoria ternatea* flower extract, (2) green synthesis of AuNPs under different reaction times, and (3) physicochemical characterization of the synthesized nanoparticles using UV-Vis spectroscopy, FTIR spectroscopy, and SEM analysis.

The independent variable was the reaction time (0, 30, 60, 90, and 120 min), while the dependent variables included the optical properties, functional groups, and morphology of the synthesized AuNPs. All treatments were conducted in triplicate to ensure reproducibility and reliability of the experimental results.

A schematic illustration of the experimental workflow is presented in Figure 1.

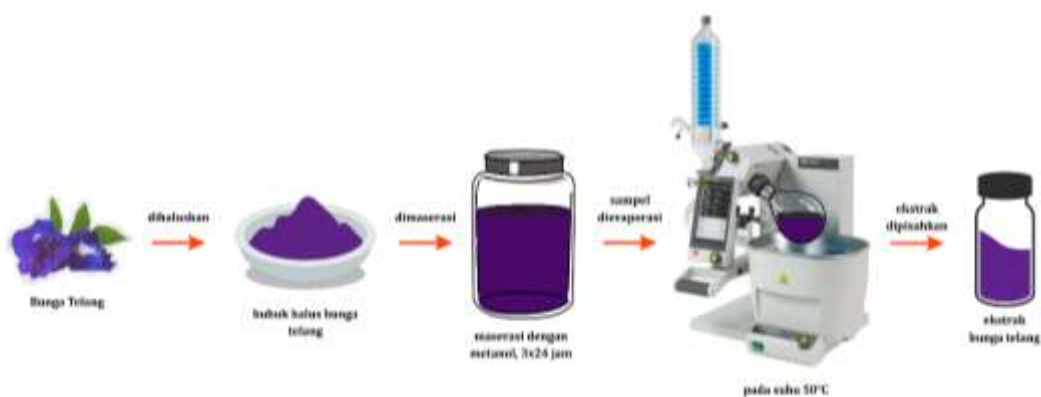


Figure 1. Schematic representation of the extraction process of *Clitoria ternatea* flowers.

Preparation of Butterfly Pea Flower Extract

Fresh flowers of *Clitoria ternatea* were collected from cultivation areas in South Sulawesi, Indonesia. The samples were thoroughly rinsed with running

water to eliminate adhering dust and impurities before being air-dried at ambient temperature ($27 \pm 2^\circ\text{C}$) for approximately 5–7 days until a constant weight was reached.

The dried *Clitoria ternatea* flowers were pulverized into a fine powder using a laboratory blender. Approximately 50 g of the powdered material was extracted with 500 mL of methanol (1:10, w/v) by maceration for 72 h at room temperature. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 50°C using a rotary evaporator. The resulting crude extract was stored at 4°C until it was used for the green synthesis of gold nanoparticles.

Synthesis of Gold Nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) were synthesized via a green synthesis approach using *Clitoria ternatea* flower extract as the natural reducing and stabilizing agent. A 3 mM chloroauric acid (HAuCl_4) solution was prepared by dissolving an appropriate amount of HAuCl_4 in distilled water. Subsequently, 9 mL of the HAuCl_4 solution was combined with 1 mL of the flower extract to obtain a precursor-to-extract ratio of 9:1 (v/v), following the procedure reported by Saputra et al., (2020).

The reaction mixture was continuously stirred at 500 rpm under ambient laboratory conditions ($27 \pm 2^\circ\text{C}$). The formation of AuNPs was indicated by a gradual color change from pale yellow to ruby red, reflecting the reduction of Au^{3+} ions to elemental gold (Au^0) and the development of the characteristic Surface Plasmon Resonance (SPR). To investigate the effect of reaction time on nanoparticle formation and physicochemical properties, aliquots of the reaction mixture were collected at 0, 30, 60, 90, and 120 min for subsequent characterization (Negara et al., 2026).

RESULTS AND DISCUSSION

Successful formation of AuNPs was initially verified through the distinct visual transformation of the reaction solution from pale yellow to a characteristic ruby-red color. This observation indicates that phytochemical constituents contained in *C. ternatea* extract effectively reduced Au^{3+} ions into metallic Au^0 nanoparticles. The emergence of the ruby-red coloration is associated with localized surface plasmon resonance (LSPR), which is widely recognized as a characteristic optical signature of dispersed gold nanoparticles rather than dissolved gold ions. (Kumalasari et al., 2024; Veeraragavan et al., 2025).

FTIR Analysis of Clitoria ternatea Flower Extract

FTIR was employed to investigate the phytochemical functional groups present in *C. ternatea* flower extract that are potentially responsible for the reduction and stabilization of gold nanoparticles during the green synthesis process. The recorded FTIR spectrum (Figure 2) together with the corresponding band assignments (Table 1) demonstrates the presence of several oxygen-containing and aromatic functional groups commonly associated with plant-derived secondary metabolites.

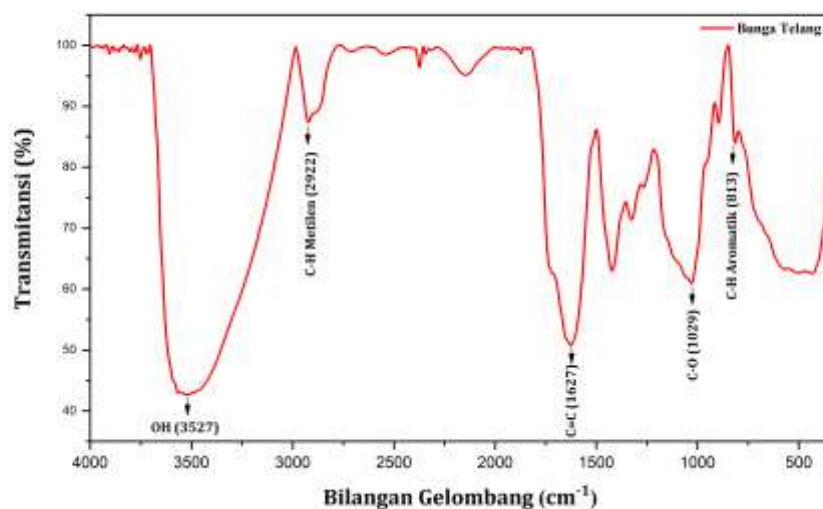


Figure 2. FTIR spectrum of *Clitoria ternatea* flower extract.

Table 1. FTIR absorption bands and functional group assignments of *Clitoria ternatea* flower extract.

No.	Wavenumber (cm ⁻¹)	Functional Group	Reference Wavenumber (cm ⁻¹)	Reference
1	813	Aromatic C-H	860–800	(Nandiyanto et al., 2019)
2	1029	C-O	1270–1230	(Nandiyanto et al., 2019)
3	1627	C=C	1625	(Nandiyanto et al., 2019)
4	2922	Methylene C-H	2935–2915	(Nandiyanto et al., 2019)
5	3527	O-H	3570–3200	(Nandiyanto et al., 2019)

A broad absorption band centered at approximately 3527 cm⁻¹ was assigned to O-H stretching vibrations, indicating the abundance of hydroxyl groups originating from phenolic compounds, flavonoids, and anthocyanins. These hydroxyl-containing molecules are considered the primary electron donors during nanoparticle synthesis because they facilitate the conversion of Au³⁺ ions into metallic Au⁰ through oxidation of their hydroxyl functionalities. Consequently, these phytochemicals play a dual role as both reducing agents and surface-protecting molecules throughout nanoparticle formation.

The absorption peak observed at 2922 cm⁻¹ corresponds to aliphatic C-H stretching vibrations, suggesting the presence of hydrocarbon chains associated with various organic constituents in the extract. Another prominent band located at 1627 cm⁻¹ was attributed to aromatic C=C stretching vibrations, confirming the

existence of conjugated aromatic structures typical of polyphenolic compounds. Such conjugated systems enhance electron delocalization, thereby facilitating electron transfer reactions involved in the reduction of gold ions.

In addition, the absorption band at approximately 1029 cm^{-1} was assigned to C–O stretching vibrations of alcohols, ethers, or glycosidic linkages, whereas the band around 813 cm^{-1} corresponds to aromatic C–H out-of-plane bending vibrations. These spectral features further support the presence of flavonoid-derived compounds and other oxygenated metabolites that actively participate in nanoparticle formation.

Overall, the FTIR results confirm that *C. ternatea* extract contains abundant phytochemicals capable of mediating AuNP synthesis without the need for synthetic reducing agents. Besides promoting the reduction of Au^{3+} ions, these biomolecules are adsorbed onto the nanoparticle surface, forming a protective organic layer that provides steric and electrostatic stabilization. Such interactions effectively suppress excessive particle aggregation and contribute to improved colloidal stability of the synthesized gold nanoparticles. These observations are consistent with previous reports describing the essential role of plant-derived polyphenols in the green synthesis and stabilization of noble metal nanoparticles (Georgeous et al., 2024; Sharma et al., 2025).

Synthesis of Gold Nanoparticles

The successful biosynthesis of AuNPs using *C. ternatea* flower extract was verified by UV–Visible spectroscopy, which revealed the emergence of a distinct absorption band within the 500–600 nm wavelength region (Figure 3). This spectral feature corresponds to the localized SPR of AuNPs and serves as a reliable indicator of nanoparticle formation. The observed SPR behavior agrees with previous reports describing the optical characteristics of biologically synthesized gold nanoparticles (Ahmed et al., 2022; Saputra et al., 2020).

At the initial reaction time (0 min), the UV–Vis spectrum showed no characteristic SPR absorption, indicating that the reduction of Au^{3+} ions had not yet progressed to an appreciable extent. Following 30 min of reaction, a weak but discernible SPR band appeared, accompanied by an increase in absorbance intensity. This observation suggests that the phytochemicals present in *C. ternatea* extract had initiated the reduction process, leading to the formation of the first stable Au nuclei. During this stage, nucleation is expected to dominate over particle growth, resulting in a relatively low concentration of nanoparticles dispersed in the reaction medium

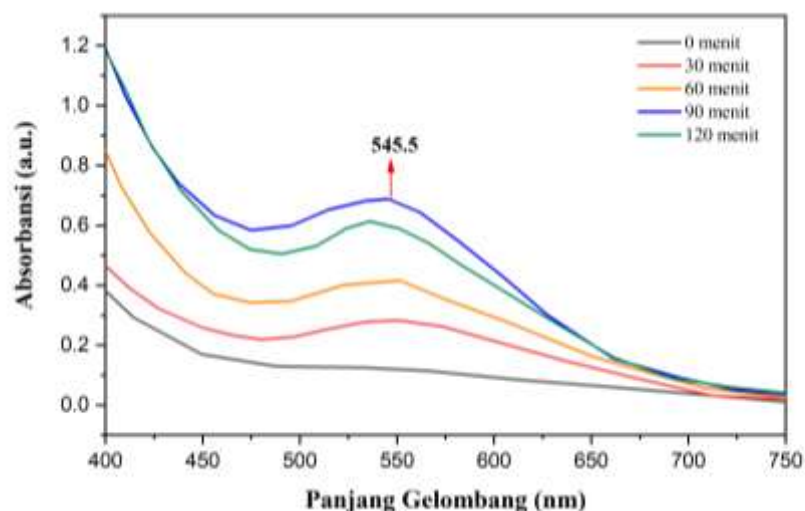


Figure 3. UV-Vis spectra of gold nanoparticles (AuNPs) synthesized using *Clitoria ternatea* flower extract at different reaction times.

As the reaction proceeded to 60 min, the absorbance increased substantially, reflecting the continuous conversion of Au^{3+} ions into metallic gold and the subsequent enlargement of previously formed nuclei. The progressive increase in absorbance indicates that nanoparticle growth became the predominant process after the initial nucleation stage. Simultaneously, the SPR band became more intense, demonstrating the increasing population of colloidal dispersed AuNPs.

The highest absorbance intensity was recorded after 90 min of reaction, while the SPR maximum remained centered at approximately 545.5 nm. This result suggests that the reduction process had reached its optimum stage, producing a greater number of nanoparticles with relatively homogeneous dimensions. The absence of a significant wavelength shift indicates that particle size distribution remained comparatively uniform throughout the synthesis, while the increased peak intensity reflects enhanced nanoparticle yield rather than substantial changes in particle morphology.

Extending the reaction time to 120 min resulted in a slight decline in absorbance despite the persistence of the SPR peak. This behavior may indicate that most Au^{3+} ions had already been consumed and that the reaction approached equilibrium. Furthermore, prolonged reaction periods may promote limited particle aggregation or Ostwald ripening, both of which can reduce the effective concentration of well-dispersed nanoparticles without eliminating the characteristic SPR response.

Overall, the UV-Vis results demonstrate that reaction time strongly influences the kinetics of AuNP formation by regulating nucleation, particle growth, and colloidal stability. Among the investigated conditions, a reaction time of **90 min** provided the most favorable optical characteristics, suggesting that this duration represents the optimum balance between reduction efficiency and nanoparticle stabilization. The stable SPR position observed throughout the

synthesis further indicates that the phytochemical constituents of *C. ternatea* extract effectively functioned not only as reducing agents but also as natural capping agents that maintained nanoparticle dispersion and minimized excessive aggregation (Assad et al., 2025; Parfenova et al., 2025).

SEM Analysis of Gold Nanoparticles

Scanning Electron Microscopy was employed to evaluate the surface morphology and microstructural characteristics of the biosynthesized gold nanoparticles. As presented in Figure 4, the obtained particles exhibited nanoscale dimensions with non-uniform morphologies, consisting predominantly of semi-spherical and irregular polygonal structures. The diversity in particle shape suggests that nanoparticle formation occurred through a dynamic nucleation and growth process influenced by the complex composition of phytochemicals present in *C. ternatea* flower extract.

The SEM micrographs further revealed that the nanoparticles were distributed across the sample surface without forming a completely homogeneous arrangement. Although individual particles were clearly distinguishable, several localized clusters were observed, indicating a moderate degree of agglomeration. Such aggregation is commonly encountered in plant-mediated nanoparticle synthesis because biomolecules adsorbed on neighboring particle surfaces may promote interparticle interactions during solvent evaporation and sample preparation for SEM analysis (Konstanty & Tyrala, 2024; Pinlova & Nowack, 2024).

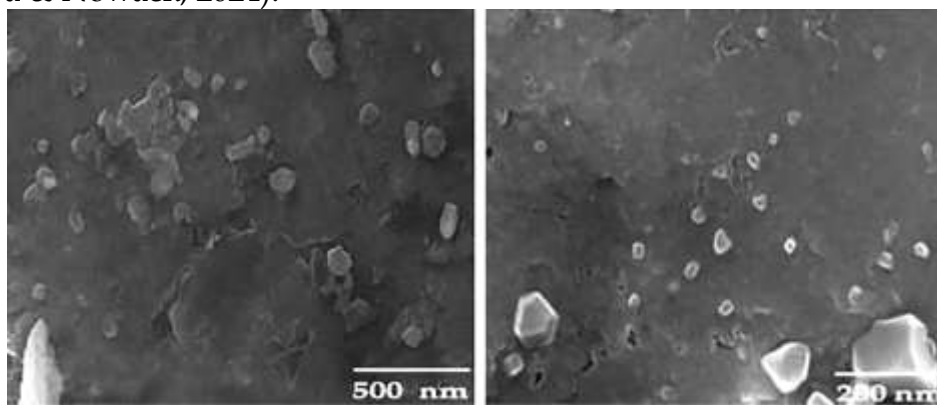


Figure 4. SEM image of gold nanoparticles (AuNPs) synthesized using *Clitoria ternatea* flower extract.

Variations in particle morphology and size can be attributed to differences in the rates of nucleation and crystal growth throughout the synthesis process. During the initial stage, rapid nucleation generates numerous small particle nuclei, whereas prolonged reaction allows these nuclei to grow into larger crystallites with more diverse geometrical features. In addition, the heterogeneous composition of reducing metabolites, including flavonoids, anthocyanins, and other phenolic compounds, may lead to different growth rates on specific crystal facets, thereby producing particles with varied morphologies instead of perfectly spherical structures (Velgosova et al., 2025; Vinukonda et al., 2025).

Despite the presence of slight agglomeration, the overall morphology demonstrates that the phytochemical constituents of *C. ternatea* extract effectively acted as natural stabilizing agents during nanoparticle synthesis. The adsorption of these biomolecules onto the AuNP surface likely reduced excessive particle coalescence by providing steric hindrance and electrostatic repulsion. Nevertheless, complete stabilization was not achieved, as evidenced by the occurrence of several aggregated domains, suggesting that intermolecular interactions between capped nanoparticles remained possible, particularly after extended reaction periods.

The SEM observations are consistent with the UV-Visible spectroscopic results, which indicated successful nanoparticle formation through the appearance of a characteristic Surface Plasmon Resonance (SPR) band. Collectively, these findings confirm that *C. ternatea* flower extract functions efficiently as both a reducing and stabilizing agent, enabling the production of gold nanoparticles with nanoscale dimensions and reasonably good morphological stability through an environmentally benign synthesis route.

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrates that *Clitoria ternatea* flower extract can be effectively utilized as a natural reducing and stabilizing agent for the environmentally friendly synthesis of gold nanoparticles (AuNPs). The formation of AuNPs was successfully verified by UV-Vis spectroscopy through the appearance of a characteristic Surface Plasmon Resonance (SPR) absorption band centered at approximately 545.5 nm, with the highest absorbance recorded after 90 min of reaction. FTIR analysis revealed the involvement of hydroxyl, aliphatic C-H, aromatic C=C, and C-O functional groups, confirming the contribution of phytochemical constituents to both the reduction of Au³⁺ ions and nanoparticle stabilization. SEM observations further confirmed the production of nanoscale particles exhibiting heterogeneous morphologies with slight agglomeration. Overall, the findings indicate that reaction time significantly influences nanoparticle formation and suggest that *C. ternatea* extract represents a promising, sustainable alternative for the green production of AuNPs intended for future nanotechnology applications.

FURTHER STUDY

Further research is recommended to investigate the effects of additional synthesis parameters, such as extract concentration, pH, temperature, and precursor concentration, on the formation and stability of gold nanoparticles (AuNPs) synthesized using *Clitoria ternatea* extract. Future studies may also evaluate the biological activities, catalytic performance, and potential applications of the synthesized AuNPs in biomedical, environmental, and industrial fields. Moreover, advanced characterization techniques and optimization approaches are suggested to better understand the mechanisms involved in green nanoparticle synthesis and to improve the scalability of environmentally friendly production methods.

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