



Synthesis AuNPs Using *Moringa oleifera* Extract and Potential Study as Colorimetric Microplastic Detection

Muhammad Bakhru Thohir*, Dyah Setyaningrum, Meilisa Rusdiana Surya Efendi, Arya Ananda Saputra, Marta Citra Nursaida

Chemistry Program, Faculty of Science and Engineering, Universitas Bojonegoro. Lettu Suyitno Street No. 2, Sukorejo, Bojonegoro, Indonesia 62119

* Corresponding Author e-mail: bakhru@unigoro.ac.id

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Abstract

Nanoparticles are multipurpose materials that have been utilized in the medical, energy, and environmental monitoring fields. The advantage of nanoparticles is that they have unique physicochemical properties such as surface area, optical activity, and surface modifiability. One of the important uses of nanoparticles is for environmental monitoring. This is because the surface of nanoparticles can be modified, and with their small size, they can reach analytes in difficult matrices. However, conventional synthesis methods of nanoparticles have been unsustainable. Therefore, the synthesis of AuNPs using bioreductors was considered urgent. The aim of this research was to determine the optimum conditions for the synthesis of AuNPs with *Moringa oleifera* (MO) as a bioreductor to obtain AuNPs-MO, to characterize the synthesized AuNPs-MO, and to study the application of AuNPs-MO for monitoring microplastic pollutants. This research was conducted through the stages of extraction, determination of optimum conditions, characterization, and literature study of the potential of AuNPs-MO as a detector. Determination of optimum conditions was carried out by applying variations in pH and precursor-reducing agent ratio. The optimal pH was found to be 6, and the optimal volume ratio was 15:5 (mL). Characterization of AuNPs-MO was conducted using FTIR and PSA. The FTIR spectra showed identical absorption patterns for AuNPs synthesized with bioreductants, and the particle size was found to be 61.15 nm. In addition, microplastics were detected using AuNPs both directly, through surface modification with proteins, and indirectly, with the assistance of acetone. From this series of experiments, satisfactory results were obtained.

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INTRODUCTION

Microplastics are among the most hazardous pollutants and have not yet been managed effectively (Aragaw, 2021). While the full extent of their risks is still being uncovered, some established impacts include lipid aggregation in organisms (Liu et al., 2023). This is not surprising, as microplastics retain several properties from their macro forms, such as being lipophilic (Yuan & Xu, 2023). Moreover, due to their microscopic size, microplastics possess a high mobility within ecosystems, further increasing their potential harm (Shamskhany et al., 2021).

Microplastics also have the ability to adsorb metals onto their surfaces. These metals adhere to plastic particles and enter biological systems through ingestion (Chen et al., 2023). Additionally, microplastics can accumulate in organisms, with their harmful effects potentially manifesting only in the long term. Therefore, the presence of microplastics and their interaction with metals underscores the importance of continuous environmental monitoring (Thohir et al., 2022; Zhu et al., 2024).

To date, microplastic analysis has primarily relied on sophisticated instruments such as FTIR and Raman spectroscopy. These techniques require professional technician, are costly, and consume significant energy (B. N. V. Kumar et al., 2021). In contrast, nanoparticles—such as gold nanoparticles (AuNPs)—offer promising alternatives as microplastic detection agents. Defined as particles ranging in size from 1 to 100 nm, nanoparticles have played a crucial role in recent scientific and technological advancements (Bayda et al., 2019; Thohir & Tiyas, 2024). AuNPs are particularly attractive for this application due to their biocompatibility, multifunctionality, high stability, and ease of surface modification (Mikhailova, 2021).

One common method for synthesizing AuNPs is chemical reduction. This involves reducing Au^{3+} ions to elemental gold (Au^0), followed by nucleation and particle growth to form nanoscale materials (Sangwan & Seth, 2022). Frequently used reductants in this process include citric acid and ascorbic acid (Poklepovich-Caride et al., 2022; Waragai et al., 2021), whose optimal conditions for synthesis have been well studied (Oliveira et al., 2023). However, reliance on synthetic materials often involves non-renewable resources. As such, there is a pressing need to explore renewable alternatives with known synthesis parameters (Ganesh et al., 2021), especially when aiming for environmentally sustainable nanomaterials.

Moringa oleifera is one such renewable candidate, known for its high ascorbic acid content. This plant offers a natural alternative to synthetic ascorbic acid, another organic acid, and also contains numerous secondary metabolites that may act as stabilizers (Perumalsamy et al., 2024). In conventional AuNP synthesis, stabilizing agents are often required in addition to the reductants; however, *Moringa oleifera* could fulfill both roles simultaneously (Ali et al., 2021). This dual function not only supports the use of renewable resources but also reduces the total amount of reagents needed (Vijayaram et al., 2024).

Previous studies have reported successful green synthesis of AuNPs using plant extracts such as *Curcuma pseudomontana*, *Capsicum annum*, *Garcinia kola*, and *Zingiber officinale* (Akintelu et al., 2021; Fouda et al., 2022; Muniyappan et al., 2021; Patil et al., 2023). However, to date, these green-synthesized AuNPs have primarily been utilized for antioxidant, antibacterial, and anticancer applications (El-Borady et al., 2020). No studies have reported the use of green-synthesized AuNPs for microplastic detection. Therefore, the state of the art of this research lies in utilizing green-synthesized AuNPs as a microplastic detection agent.

Therefore, this study aims to determine the optimum synthesis conditions of AuNPs using *Moringa oleifera* extract as a substitute for synthetic ascorbic acid by varying pH and extract-to-metal ratios. The study also seeks to characterize the resulting AuNPs-MP complex and investigate its potential in colorimetric microplastic detection.

METHOD

Material and Methods

The equipment used in this study included a UV-Vis spectrophotometer, Fourier Transform Infrared Spectrometer (FTIR), Particle Size Analyzer (PSA), hot plate, thermometer, stopwatch, and standard laboratory glassware. The materials used were *Moringa oleifera* powder, distilled water, pure gold, hydrochloric acid (HCl), and nitric acid (HNO_3).

Extraction of *Moringa oleifera*

A total of 100 mL distilled water was placed in a beaker and heated on a hot plate until boiling, with continuous stirring. Then, 0.25 g of *Moringa oleifera* powder was added to the boiling water and stirred occasionally for 15 minutes. Afterward, the solution was cooled to room temperature and filtered using filter paper to separate the residue and the extract, resulting in a

brownish-yellow *Moringa oleifera* extract. The extract was stored at 4 °C and used within two weeks; beyond this period, it must be regenerated.

Preparation of HAuCl₄ Precursor

To prepare a 0.3 mM HAuCl₄ solution, 0.1 g of pure gold was soaked in a mixture of concentrated HCl and HNO₃ (37% and 65%, respectively) in a 3:1 ratio for 48 hours in a fume hood. The mixture was then heated to obtain solid HAuCl₄. The resulting solid was dissolved in distilled water and diluted to the 100 mL mark in a volumetric flask to achieve a 3 mM solution.

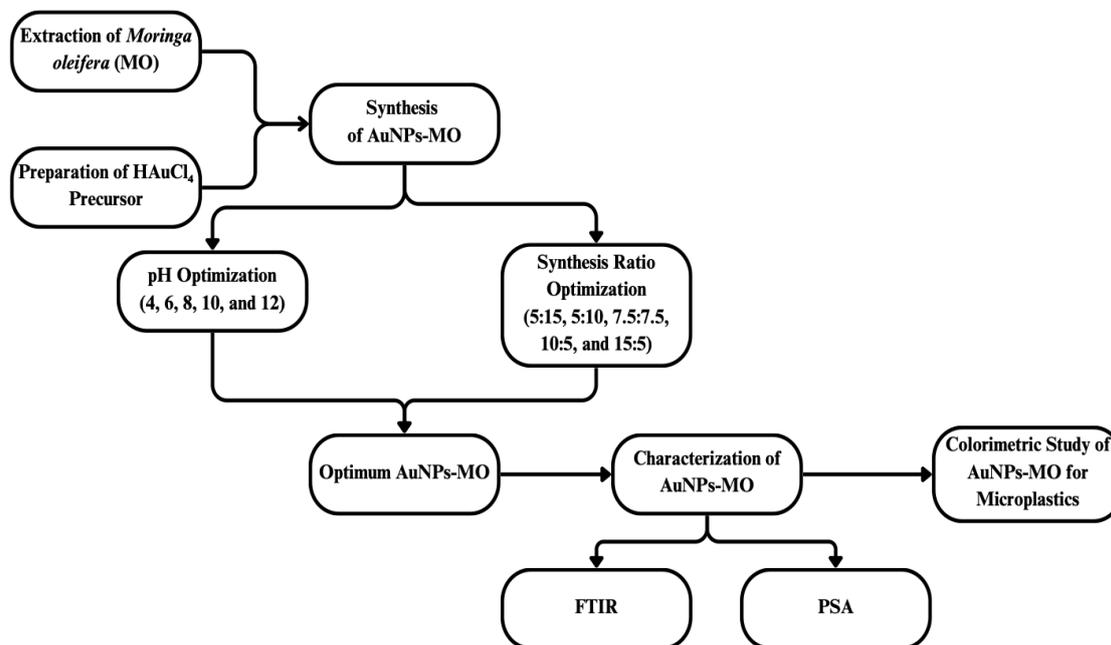


Figure 1. Research Procedure

Synthesis of AuNPs-MO: pH Optimization

The pH optimization for AuNPs-MO synthesis was performed at pH values of 4, 6, 8, 10, and 12. First, 10 mL of 0.3 mM HAuCl₄ solution was heated to boiling with continuous stirring. Then, 5 mL of *Moringa oleifera* extract (0.25% concentration), previously adjusted to the desired pH, was quickly added. The reaction was maintained under continuous stirring for 10 minutes. After heating, stirring was continued without heat until the solution reached room temperature to allow optimal AuNPs-MO formation. The synthesized AuNPs-MO were stored at 4 °C.

AuNPs-MO Synthesis: Synthesis Ratio Optimization

To optimize the synthesis ratio, *Moringa oleifera* extract was used as the bioreductant under the same procedure as in the pH optimization, using the previously determined optimal pH. The tested volume ratios of 0.3 mM HAuCl₄ solution to *Moringa oleifera* extract were 5:15, 5:10, 7.5:7.5, 10:5, and 15:5.

Characterization of AuNPs-MO

The synthesized AuNPs-MO were characterized using UV-Vis spectrophotometry to determine the maximum absorbance wavelength within the range of 400–800 nm. FTIR spectroscopy was performed to identify the functional groups of the bioreductant and the interactions between these groups. FTIR analysis was carried out in the wavenumber range of 4000–800 cm⁻¹. Additionally, particle size distribution was analyzed using a Particle Size Analyzer (PSA) to determine the size of the synthesized nanoparticles.

RESULTS AND DISCUSSION

Gold nanoparticles (AuNPs) are versatile materials that are widely used across various fields, including medicine, technology, and environmental monitoring. Their broad applicability is largely attributed to their unique physicochemical properties. AuNPs possess magnetic characteristics, tunable surface chemistry, and size-dependent optical properties, among others (Altammar, 2023). These properties enable their use in multiple applications, such as drug delivery systems that can be guided by external magnets (W. Li et al., 2019), surface modification with functional groups for adsorption purposes (Mitomo & Ijoro, 2021), and tuning of optical responses based on particle size (X. Li et al., 2021).

AuNPs are relatively easy to synthesize, and their size and shape can be precisely controlled to suit specific applications. However, the physical and chemical properties of the resulting nanoparticles are strongly influenced by their morphological characteristics. As a result, optimizing synthesis conditions is crucial to achieving desirable outcomes. Among the key parameters, the concentration of hydrogen ions (H^+) and the ratio between the gold precursor and the reducing agent play particularly important roles, as they directly influence the nucleation and growth of the nanoparticles (Hammami & Alabdallah, 2021; Yazdani et al., 2021).

Synthesis of AuNPs: determination of optimum pH

The pH range used in this study was from 4 to 12, aiming to evaluate synthesis conditions across environments with varying H^+ concentrations, from highly acidic (low pH) to strongly alkaline (high pH).

The experiment began with heating the gold precursor to increase kinetic energy, ensuring sufficient energy for the precursor to undergo reduction (Yang et al., 2021). Without external energy input, the reaction might not occur or may proceed very slowly due to insufficient activation energy (Akintelu et al., 2021). During heating, continuous stirring was applied to promote uniform particle growth by improving collision frequency. Once the precursor reached boiling point, the *Moringa oleifera* extract—preconditioned at pH values ranging from 4 to 12—was rapidly added. The reaction was maintained for 10 minutes at elevated temperature, followed by 10 minutes of stirring during cooling at room temperature to enhance nanoparticle formation (Patil et al., 2023).

As shown in Figure 2, AuNPs synthesized at pH 4 were successfully formed, as evidenced by the appearance of a wine-red color and an absorption peak (λ_{max}) at 550 nm (I. Kumar et al., 2019). However, the UV-Visible spectra indicated low homogeneity, with a broad and slightly irregular peak shape (Janah et al., 2022), suggesting polydispersity. At pH 6, AuNPs formed in greater quantity with more uniform size distribution, as shown by a sharper and narrower absorption peak and a slight blue shift to 540 nm, indicating smaller particle size.

In contrast, at pH 8, the nanoparticles formed were significantly fewer, demonstrated by a lower absorption intensity. The spectrum displayed a broader peak and the solution color was no longer wine-red, implying reduced nanoparticle formation. Nevertheless, a small absorption peak at 500 nm still suggests the presence of some very small-sized AuNPs (Ahmad et al., 2022).

When the pH increased further to 10 and 12, no clear formation of AuNPs was observed. The solutions displayed a yellow-orange color, often associated with nanoparticle aggregation or unsuccessful reduction. At pH 12, the presence of a visible precipitate confirmed coagulation, supported by the absence of characteristic AuNP peaks in the UV-Visible spectra.

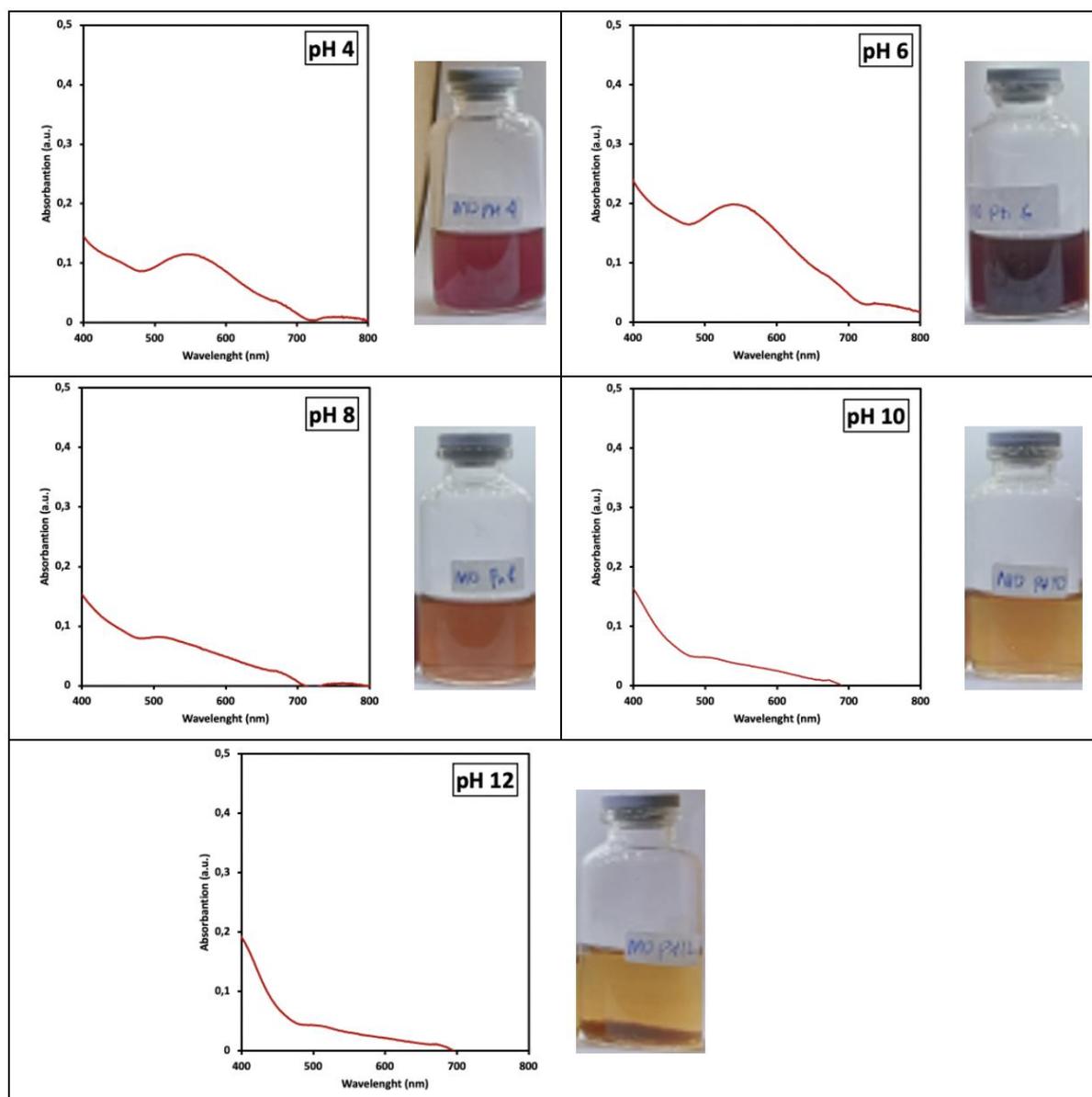


Figure 2. Synthesis Results of AuNPs-MO with Variations in pH of Reducing Agents. (left) UV-visible spectra of synthesis results and (right) visual appearance of AuNPs-MO.

These results suggest that AuNPs are optimally synthesized under acidic conditions, particularly at pH 6. Under acidic pH, gold predominantly exists as the AuCl_4^- complex, which is readily reduced to Au^0 . In contrast, under alkaline conditions, ligand substitution reactions occur, where Cl^- is replaced by OH^- to form complexes such as $\text{AuCl}_3(\text{OH})$, $\text{AuCl}_2(\text{OH})_2$, $\text{AuCl}(\text{OH})_3$, and $\text{Au}(\text{OH})_4^-$, which are more resistant to reduction and prone to precipitation (Pranata Putri et al., 2024).

Reduction pathways at different pH values:

At acidic condition : $\text{AuCl}_4^- + \text{C}_6\text{H}_8\text{O}_6^{2-} \rightarrow \text{Au}^0$ (Au is reduced, then nucleates and forms AuNPs)

At alkaline condition : $\text{AuCl}_3(\text{OH}) + \text{C}_6\text{H}_8\text{O}_6^{2-} \rightarrow \text{X}$
 $\text{AuCl}_2(\text{OH})_2 + \text{C}_6\text{H}_8\text{O}_6^{2-} \rightarrow \text{X}$
 $\text{AuCl}(\text{OH})_3 + \text{C}_6\text{H}_8\text{O}_6^{2-} \rightarrow \text{X}$
 $\text{Au}(\text{OH})_4 + \text{C}_6\text{H}_8\text{O}_6^{2-} \rightarrow \text{X}$

There is no reduction occurred due to gold precipitation and a weak reducing environment.

In addition, ascorbic acid contained in *Moringa oleifera* extract becomes a reactive species for reduction at low pH. Ascorbic acid in acidic conditions exists in the form of $C_6H_8O_6$ and $C_6H_7O_6^-$. The $C_6H_8O_6$ species exists in greater quantities, but the most reactive one working as a bioreductant is $C_6H_7O_6^-$. Therefore, from here it can be seen that the acidic condition of the Au precursor is more ready to be reduced, and ascorbic acid is also more ready to reduce. While in alkaline conditions, ascorbic acid is in a more unstable and damaged $C_6H_7O_6^{2-}$ species condition so that it fails to reduce. In addition, the Au species that does not have a Cl ligand and has been replaced with OH will make electron transfer slow, because the one that acts as an electron bridge (bridge connection) is the Cl species (Pranata Putri et al., 2024).

Synthesis of AuNPs: Ratio of Precursor and Reducing Agent

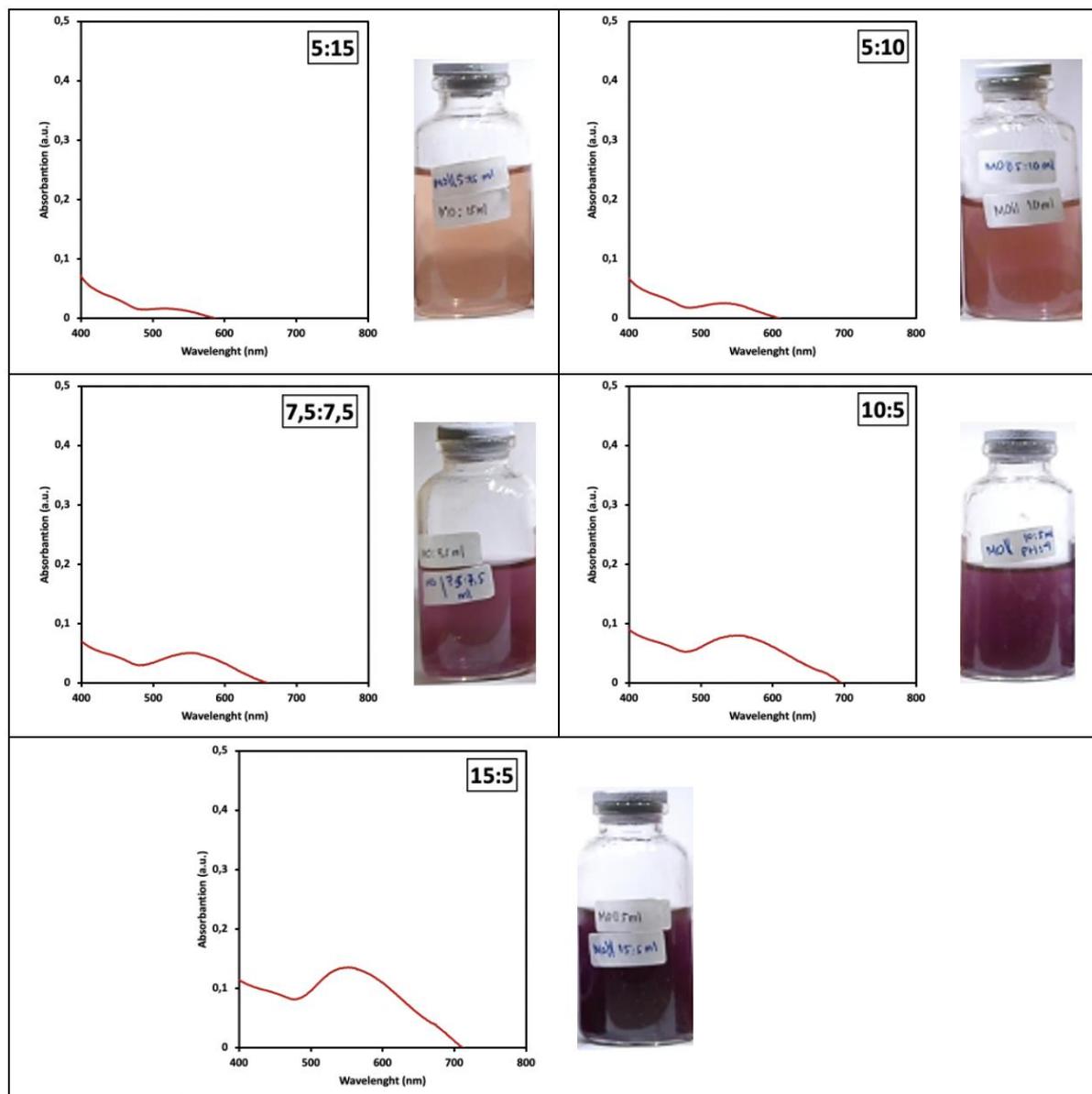


Figure 3. Synthesis Results of AuNPs-MO with Variations in the Ratio of Precursors to Reducing Agents. (left) UV-visible spectra of the synthesis results and (right) visual appearance of AuNPs-MO.

This experiment followed the same procedure as the previous stage, with the only difference being the variation in the volume ratio between the gold precursor and the reducing agent. The

pH of the reducing agent was maintained at 6, based on the previously determined optimal condition. The ratios tested were 5:15, 5:10, 7.5:7.5, 10:5, and 15:5 (mL).

The results are presented in Figure 3. At a 5:15 ratio, where the amount of reducing agent was much greater than the precursor, only a small amount of AuNPs was formed. This was indicated by the physical appearance of the solution, which turned a bright red color, and by the UV-visible spectrum, which showed a low absorbance peak at 530 nm (Fouda et al., 2022). As the amount of precursor increased, the formation of AuNPs-MO also increased. This was evidenced by the more intense wine-red color of the solution and higher absorbance at 540 nm in the UV-visible spectra, indicating a higher concentration of AuNPs.

These results indicate that the reducing ability of *Moringa oleifera* extract is very strong (Perumalsamy et al., 2024). At higher precursor concentrations, aggregation did not occur, indicating stable nanoparticle formation. However, when the amount of precursor exceeded that of the reducing agent, aggregation and bridging between nanoparticles were observed. This may be due to the presence of unreacted Au precursors, which remain in the form of AuCl_4^- (El-Khawaga et al., 2023).

Further analysis shows that an excessive amount of reducing agent—such as in the 5:15 ratio—can act as a steric hindrance, inhibiting effective interaction with the gold precursor. This could explain the low AuNPs yield in that condition (Pasiczna-Patkowska et al., 2025). Based on these findings, the 15:5 ratio was identified as the most optimal, despite resulting in slightly larger nanoparticle sizes (indicated by a redshift in λ_{max}). This ratio produced a higher concentration of AuNPs with better homogeneity compared to the other ratios tested.

Characterization of AuNPs-MO

AuNPs-MO synthesized at pH 6 using a precursor-to-reducing agent ratio of 15:5 were characterized by FTIR and PSA, as shown in Figures 4 and 5.

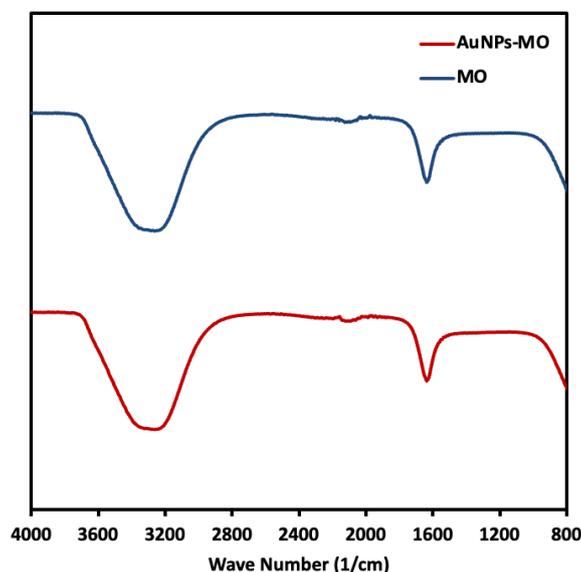


Figure 4. FTIR spectra (red) of AuNPs-MO synthesized under optimum conditions and (blue) *Moringa oleifera* extract.

The FTIR spectrum (Figure 4) displays several characteristic absorption bands, including a peak at 3265.1 cm^{-1} corresponding to O–H stretching vibrations, which are indicative of hydroxyl groups present in the *Moringa oleifera* extract (Fouda et al., 2022). A band observed at 2124.6 cm^{-1} is attributed to the stretching vibrations of either C≡C or C≡N groups, while the peak at 1636.3 cm^{-1} corresponds to symmetric stretching of the carbonyl group (C=O), all of

which originate from phytochemicals in the bioreductant (Afrin & Vickram, 2025; Garg et al., 2025). The presence of functional groups such as O–H, C=O, and C≡N indicates the involvement of biomolecules including flavonoids, phenols, and organic acids—compounds known to play a significant role in the reduction of gold precursors (van de Langerijt et al., 2023).

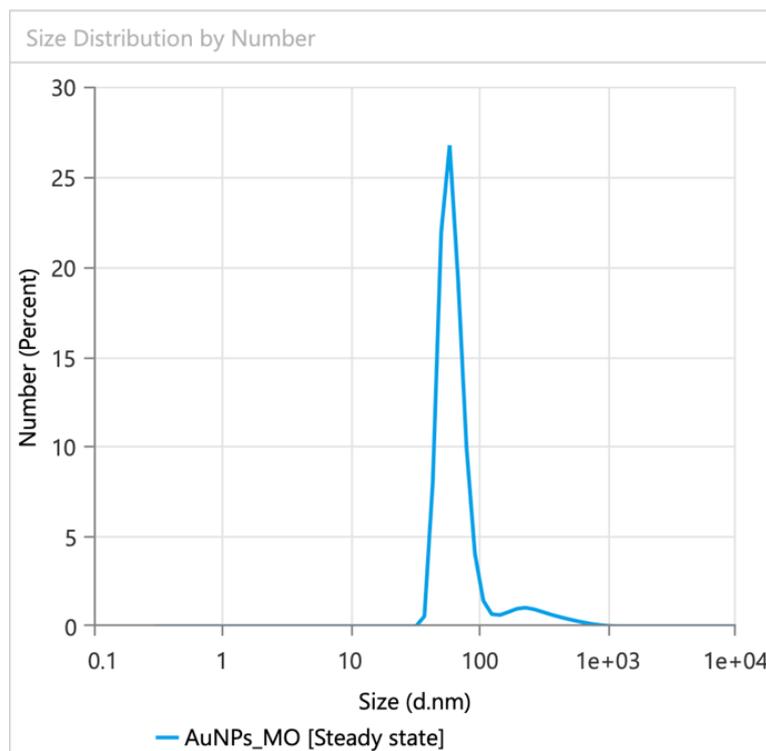


Figure 5. PSA spectra of AuNPs-MO synthesized under optimum conditions.

Table 1. PSA result data of AuNPs-MO synthesized under optimum conditions.

Data	Value (Average)
Peak 1, mean size (nm)	61.15
Peak 1, percentage size (%)	92.64
Peak 2, mean size (nm)	306.8
Peak 2, percentage size (%)	7.361

Meanwhile, the PSA analysis results (Figure 5 and Table 1) show that the synthesized AuNPs-MO have an average particle size of 61.15 nm. This size falls within the nanomaterial range (1–100 nm), confirming the nanoscale nature of the product. Furthermore, the 61.15 nm size represents the dominant particle size, accounting for 92.64% of the distribution. These findings indicate that the *Moringa oleifera* extract effectively facilitated the reduction of gold ions and the formation of stable AuNPs-MO (Fouda et al., 2022).

Colorimetric Study of AuNPs-MO for Microplastics

AuNPs exhibit unique optical properties, as their visible color can shift with changes in particle size. Additionally, AuNPs synthesized using bioreductants possess surface functionalities—particularly hydroxyl (–OH) groups—that can interact with microplastics. However, peptide-modified AuNPs demonstrate enhanced performance. Thermodifferentiated AuNPs conjugated with peptides can easily interact electrostatically with microplastics. When AuNPs-MO-Peptides are introduced into a solution, they initially appear wine red. Upon the addition of microplastics, the particles increase in size due to aggregation and exhibit a redshift in absorbance, resulting in a visible color change from wine red to purple (Zhao et al., 2023).

Another strategy for microplastic detection using synthesized AuNPs-MO involves the use of acetone as an aggregating agent. In the absence of microplastics, AuNPs aggregate upon interaction with acetone, producing a purple color. In contrast, when microplastics are present, they act as steric barriers that hinder aggregation, thus preventing the color change. As a result, the AuNPs-MO solution retains its original wine red color even after acetone is added, as illustrated in Figure 6 (Hong et al., 2022).

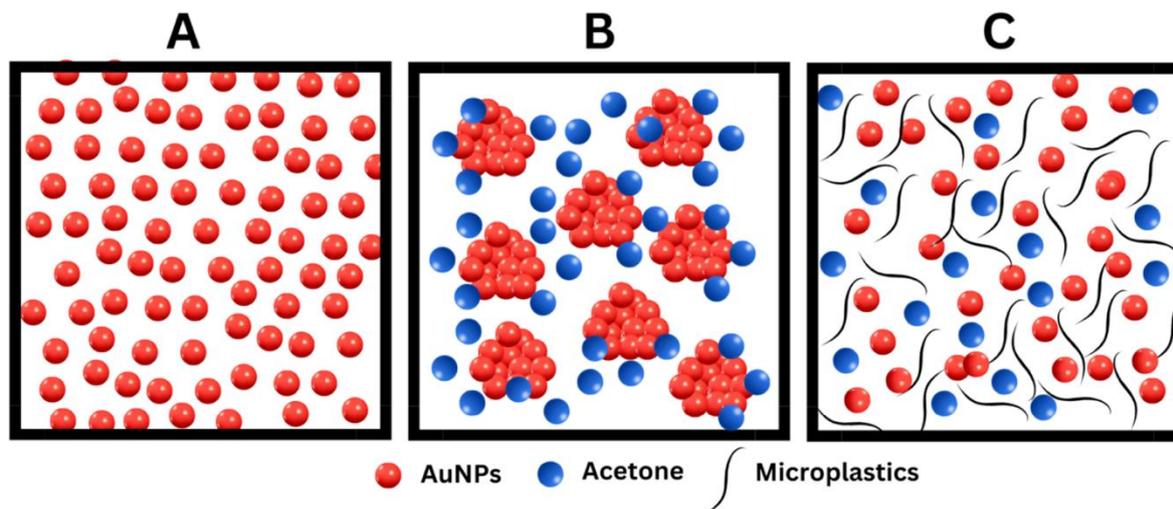


Figure 6. Simulation of the reaction of AuNPs with acetone and microplastics (A) AuNPs, (B) AuNPs aggregated due to being driven and directed by acetone, and (C) AuNPs not aggregated by acetone due to the presence of microplastics.

These findings highlight the strong potential of AuNPs-MO as a simple, rapid, and environmentally friendly platform for detecting microplastic pollutants. Nonetheless, further optimization of the synthesis process is needed to ensure consistency and reliability. Surface modification strategies may also be necessary to enhance compatibility and performance in real-world detection applications.

CONCLUSION

The synthesis of AuNPs using *Moringa oleifera* extract as a bioreductant has yielded satisfactory results. The process was optimized by adjusting the pH and the precursor-to-reductant ratio, with the optimal conditions identified at pH 6 and a ratio of 15:5 (mL). The synthesis method was straightforward and efficient. FTIR analysis confirmed the presence of functional groups corresponding to flavonoids, phenols, and organic acids, as indicated by absorption bands at 3265.1 cm^{-1} , 2124.6 cm^{-1} , and 1636.3 cm^{-1} . Particle size analysis (PSA) revealed that the synthesized AuNPs-MO had an average size of 61.15 nm, with a dominant size distribution reaching 92.64%. These findings demonstrate that AuNPs-MO have significant potential for use in microplastic detection, due to their ability to interact electrostatically with microplastics or respond to the presence of aggregating agents such as acetone.

RECOMMENDATIONS

In this research, variations in extraction and synthesis have not been discussed much, so in the future, these two things should be taken into account to find out so that the results of AuNPs synthesis are more optimal.

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