Determination of Essential Factors Affecting Silver Nanoparticle Synthesis using *Moringa oleifera* Leaves

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Abstract. The presence of secondary metabolites in *Moringa oleifera* is the decisive factor for the green synthesis of silver nanoparticles (AgNPs). Among the metabolites involved in this process, phenols and flavonoids in the plant extract are the two most important groups, acting both as reducing and stabilizing agents. *M. oleifera*, a well-known herb from Indonesia, was used in this study due to its exceptional properties, such as antioxidant, antimicrobial, and anti-inflammatory among others. The total phenolic content and total flavonoid content in the aqueous extract of *M. oleifera* leaves were determined, and the values were 62.01 ± 2.05 mg/g and 71.97 ± 0.94 mg/g, respectively. The synthesis factors including pH, extract volume, and temperature for the synthesis of AgNPs were optimized using the one-factor-at-a-time approach. The synthesis experiment showed that a pH of 8.0, an extract volume of 0.3 mL, and a temperature of 80 °C resulted in the highest intensity of localized surface plasmon resonance at a wavelength of 419 nm, which favored the synthesis after 24 h of reaction time. Due to the remarkable content of metabolites, *M. oleifera* can be used as a promising candidate for the effective synthesis of AgNPs.

1 Introduction

The green approach to the synthesis of metallic nanoparticles is part of green chemistry and technology that involves the use of plants, microbial resources, or their derivatives to synthesize materials with nanoscale dimensions. In recent years, there has been a surge of interest in the green synthesis of nanoparticles. Sugars, alkaloids, terpenoids, polyphenols, phenolic acids, and proteins play an important role in the bioreduction process along with other plant metabolites. This green synthesis method offers several advantages, such as low energy consumption and mild operating conditions without the use of harmful chemicals [3].

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Garg et al. [4] reported that various biological resources, including the use of plant extracts, *Streptomyces*, bacteria, fungi, and algae, are considered non-toxic, safe, and more environmentally friendly, and therefore, the preferred alternative to chemical and physical methods. Several studies have been conducted to determine whether a green approach for the synthesis of metal nanoparticles is simpler, cheaper, safer, and more environmentally friendly [5], [6]. The biological synthesis of nanoparticles does not produce toxic substances because natural mediators are present for the capping, reduction, and stabilization of the nanoparticles [7].

Silver is the most studied metallic nanoparticle due to its versatile applications. Many studies have shown that silver nanoparticles (AgNPs) synthesized using plants have better stability than those synthesized with microbes [1-2]. Plant extract could be used to change the size and shape of nanoparticles [8]. However, the mechanisms by which plants synthesize them are not yet clear [9]. Furthermore, it is known that plant extracts reduce metal ions faster than microbes [10]. In addition, this method is easily scalable, and plant-based synthesized nanoparticles are used in various applications for the benefit of humans. As no toxic substances are used throughout the synthesis process, the use of environmentally friendly materials, such as plant extracts in the synthesis of AgNPs, enables various applications in medicine.

Moringa oleifera leaves have higher antioxidant activity due to the presence of phenolic acids (chlorogenic acid and gallic acid) and flavonoids (rutin, quercetin, apigenin, luteolin, and kaempferol). Salicylic acid concentration in M. oleifera is also similar to that of certain fruits and vegetables, and it contains higher amounts of myricetin than spinach, while ferulic acid is similar to that of oranges, red cabbage, aubergine, and peanuts [11]. Similarly, Moodley et al. [12] reported that the leaf extract of M. oleifera showed the presence of terpenoids, flavones, and polysaccharides, which are mainly responsible for the reduction and capping of AgNPs. Saputri et al. [13] recently reported that the oil extracted from the seeds of the Moringa plant is rich in sterols, including stigmasterol, campesterol, and β-sitosterol, exhibiting anti-inflammatory properties that surpass those of rosiglitazone (commercial anti-inflammatory drug) and may therefore represent a promising avenue for the development of anti-inflammatory treatment for individuals with type 2 diabetes mellitus. The leaves of M. oleifera have a wide range of medicinal properties, including antimicrobial, antioxidant, antifertility, antipyretic, antidiabetic, antitumor activity, anti-inflammatory, cardiovascular, hepatoprotective, central nervous system, analgesic, and diuretic and wound-healing activities, as well as the ability to treat constipation, digestive, and ocular disorders [14]. The leaves are also used to treat various types of fever, sore throat, respiratory problems, hemorrhoids, scurvy, and ear and eye infections, whereas the leaf juice lowers blood sugar levels and reduces glandular swelling [15 - 17].

In this study, M. oleifera was obtained from Indonesia, and the total phenolic and flavonoid contents were quantitatively determined. The factors affecting the synthesis of AgNPs using M. oleifera leaf extract including pH, temperature, and extract volume were also optimized to obtain a high yield of AgNPs.

2 Experimentation

2.1 Chemicals and Materials

The biomass of *M. oleifera*, which was received in March 2023, was procured in powdered form as-prepared and packaged by Universal Moringa Indonesia. The biomass was stored at 4 °C. The silver nitrate ($AgNO_3$) utilized in the study was supplied by VWR International UK, while sodium hydroxide was obtained from RCI Labscan Limited. Quercetin ($C_{15}H_{10}O_7$)

was procured from Sigma-Aldrich; Folin-Ciocalteu (FC) reagent was supplied by Merck, Germany and Sigma-Aldrich; gallic acid was obtained from Sigma-Aldrich; sodium carbonate (Na_2CO_3) was acquired from VWR BDH Chemicals, Radnor, Pennsylvania, United States of America; sodium nitrate was procured from Sigma-Aldrich and Merck, Germany; and aluminum chloride ($AlCl_3$) was supplied by R&M Chemicals. Ultrapure water was produced using a Milli-Q device (model H2O-I-1-UV-T), and a pH meter (model 210) was used for the analysis. A visible spectrophotometer (model Jenway 7200) and a Memmert universal oven (model UN55) were also employed in the study.

2.2 Plant Extraction and Silver Nitrate Solution

The extraction method by Moodley *et al.* [12] was employed to obtain the extract. Approximately 100 mL of ultrapure water was used to dissolve 10 g of *M. oleifera* powder. The mixture was then stirred overnight, and the filtrate was obtained with the aid of Whatman filter paper. The resulting extract was utilized for the synthesis. The extract was divided into two portions and the pH was adjusted to pH 7 and 8. A $AgNO_3$ solution was prepared by dissolving 0.145 g/L, corresponding to a 0.85 mM solution, which was used throughout the synthesis process.

2.3 Determination of Total Phenolic Content

The FC method was used to determine the total phenolic content (TPC) in the plant extract. According to Siddhuraju and Becker [23], this method, which is based on electron transfer, degrades antioxidants by forming a blue complex. The preparation of the reagents and standards involved 20% Na_2CO_3 and the FC reagent, with gallic acid as the standard. The extract was obtained by soaking 10 g of *M. oleifera* powder in 100 mL of distilled water overnight in ultrapure water, and the TPC was calculated using the formula GAE (mg/g) = C × V/M, where GAE is gallic acid equivalent, C is the concentration obtained from a standard curve, V is the volume of extract obtained after extraction, and M is the mass of biomass.
Place the figure as close as possible after the point where it is first referenced in the text. If there is many figures and tables, it might be necessary to place some before their text citation.

2.4 Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined based on the $AlCl_3$ colorimetric method employed by Siddhuraju and Becker [22]. The flavonoids form a complex with $AlCl_3$, causing a bathochromic shift and hyperchromic effect. Reagents and standards required include Quercetin standard stock solution (5 mg/mL). The calculation of TFC is expressed as mg quercetin equivalent/g dry weight of the plant using the formula TFC (mg/g) = CV/M, where C is the concentration obtained from a standard curve, V is the volume of extract obtained after extraction, and M is the mass of biomass powder used during extraction.

2.5 Synthesis of Silver Nanoparticles

Three test tubes were carefully arranged for each pH 7 and 8. Then, 10 mL of 0.8 mM $AgNO_3$ was pipetted accurately into each tube. The extract with the pH adjusted to 7 and 8 was added to the respective sets in a range of 0.1–0.3 mL. The resulting mixture was incubated in the oven at different sets of temperatures (30, 40, 50, 60, 70, and 80 °C) for 24 h.

2.6 Optimization Process

The effects of each synthesis factor (pH, temperature, and volume) were determined based on the one-factor-at-a-time approach, and a fixed concentration of AgNO3 of 0.8 mM (0.145 mg/L) was used. Ten mL of AgNO3 solution was placed in two sets of 15 mL Falcon tubes. For pH 7, three volumes (0.1, 0.2, and 0.3 mL) of the adjusted extract were added to each tube, and a similar procedure was followed for pH 8. Two sets for each temperature (30, 40, 50, 60, 70, and 80 °C) were incubated for 24 h. For each set, the spectra were taken with a visible spectrophotometer (model Jenway 7200), and the surface plasmon resonance (SPR) was recorded.

3 Results and Discussion

The TPC and TFC of the *M. oleifera* aqueous extract were 62.01 ± 2.05 mg/g and 71.97 ± 0.94 mg/g, respectively. The variation in the content of phenolic and flavonoid compounds is subjected to various factors, such as plant variety, maturity stage, climatic conditions, plant parts, processing, and storage among others [21]. Siddhuraju and Becker [22] observed that the leaf samples collected from Nicaragua exhibited a higher concentration of total phenolics (4.25%) compared to those from India (2.94%) and Niger (3.66%) based on the samples collected from different countries. They further contended that all freeze-dried leaf samples contained the quercetin concentration ranged between 633.5 and 926 mg/100 g and the kaempferol concentration varied between 104.7 and 225.4 mg/100 g, which is higher than the concentration obtained in this report. The utilization of aqueous medium as the extraction solvent in this analysis is evidently apparent, where the metabolites extracted are less in comparison with the extraction using methanol, as declared by the same authors. In their report, Siddhuraju and Becker [22] mentioned that methanol and ethanol are superior solvents for extracting antioxidant metabolites in comparison to aqueous solvents. Additionally, the total phenolic and flavonoid contents of the methanolic extract exhibited higher values than that of the hexane extract, according to Abdulkadir et al. [23]. The authors further affirmed that the methanolic leaf had the highest phenolic content, with a value of 32.83 ± 1.38 mg/g, and the flavonoid content was 98.67 ± 2.10 mg/g, exceeding the values of the hexane extract. Therefore, the presence of phenolic and flavonoid components in *M. oleifera* can be ascertained, depending on the solvent employed in the extraction process. This result shows that *M. oleifera* is rich in both phenols and flavonoids. Susanto et al. [24], reported that *M. oleifera* contains a considerable content of phenolic compounds as well as isothiocyanates, which belong to flavonoid compounds. Flavonoid is one of the most commonly reported and predicted compounds involved in the green synthesis of silver nanoparticles [2]. These compounds are widely distributed in natural raw materials and have various biological functions.

Fig. 1 shows the Vis spectra of the AgNPs synthesized from the aqueous extract of *M. oleifera* after 24 h incubation at a fixed concentration (0.85 mM) of $AgNO_3$ at different temperatures and pHs. The metabolites present in the plant are responsible for reducing, capping, and stabilizing green synthesized metallic nanoparticles [18].

In Fig. 1, the spectra show the effect of three different factors, namely pH, temperature, and volume of the extract in the synthesis of AgNPs. The side-by-side plot of the spectra indicates the absorption bands for pH 7 and 8 at different temperatures and extract volumes. The results show that an increase in pH from 7 to 8 at constant temperature and volume leads to an increase in the spectral peak. This observation suggests that solution pH plays a crucial role in the ionization of the metabolites responsible for the reduction of Ag to AgNPs. This enhances the participation of these metabolites in the formation of nucleation and eventually reduces Ag^+ to AgNPs. Hamouda et al. [25] stated that pH plays a vital role during the

synthesis of AgNPs using *Oscillatoria limnetica*, where the authors reported that pH is responsible for modifying the net charge and functional groups of the metabolites. The synthesis of AgNPs using *M. oleifera* indicates that the increase in pH is proportional to the increase in absorbance, as shown in Fig. 1. This report is consistent with the finding of Jain and Mehata, [26], where the leaf extract of *Ocimum Sanctum* was utilized for the synthesis of AgNPs, and it was revealed that the increase in pH is associated with the increase in absorbance peak.

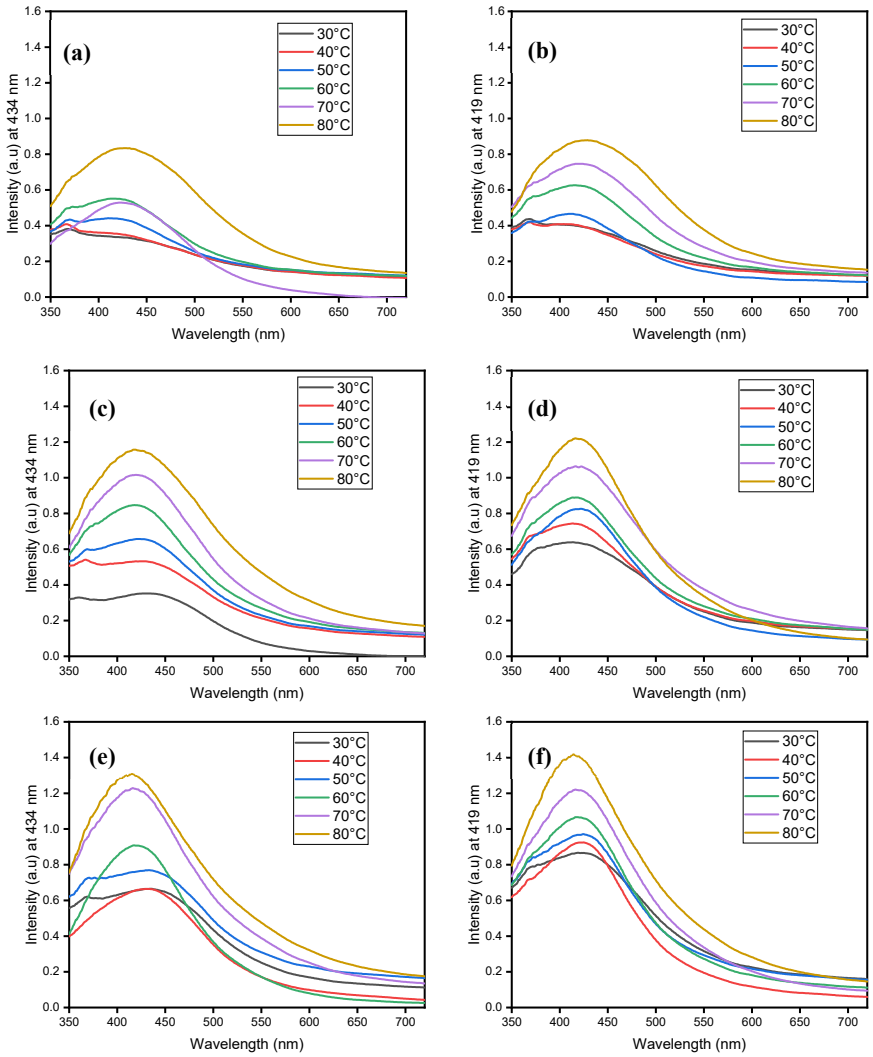

Fig. 1. Vis spectra representing synthesized AgNPs using *M. oleifera* aqueous extract with (a) extract volume of 0.1 mL at pH 7, (b) extract volume of 0.1 mL at pH 8, (c) extract volume of 0.2 mL at pH 7, (d) extract volume of 0.2 mL at pH 8, (e) extract volume of 0.3 mL at pH 7, and (f) extract volume of 0.3 mL at pH 8 at different temperatures from 30 °C to 80 °C

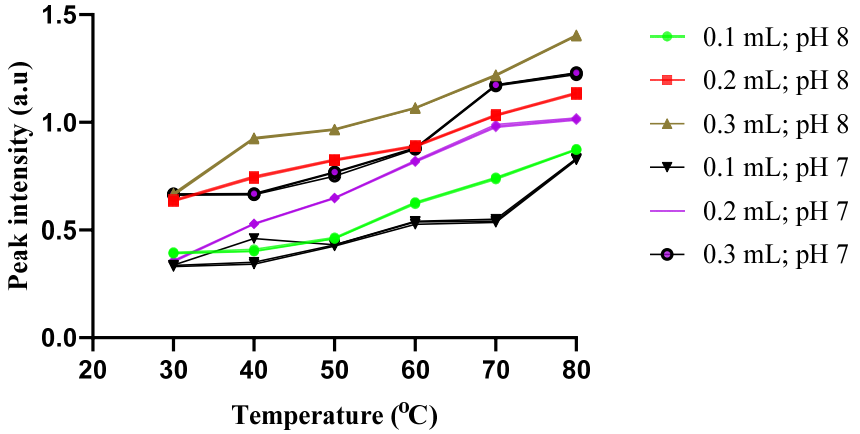

Fig. 2. Localized surface plasmon resonance intensity based on optimization with different pHs, extract volumes, and temperatures

Temperature differences play a crucial role in this study, as shown by the increase in spectral peaks as the temperature increased from 30 to 80 °C. In Fig. 2, it is clear that as the temperature increased, the absorbance also increased significantly when other factors remain constant. It is evident that the energy of the metabolites increased with temperature, thereby expediting the nucleation, reduction, and stability of AgNPs. Similarly, Hashemi et al. [27] reported that the SPR increased as the temperature increased from room temperature to 85°C. As temperature increases, new active sites are formed, thus increasing the synthesis of AgNPs. Another finding reported that temperature has a significant effect on the electrical charges of capping agents, resulting in a change in their ability to bind by reducing metal ions [28].

The importance of extract volume cannot be overstated, as an increase in extract volume leads to a corresponding increase in spectra formation. This correlation is due to the presence of metabolites in the extract, which play an important role in the reduction and stability of AgNPs. Each stage has different conditions, depending on the reducing agent concentration, pH, and $AgNO_3$. The presence of hydroxyl groups in plant biomolecules has been associated with the reduction and stabilization of Ag^+ to AgNPs [29]. The synthesis of the product is also significantly facilitated by variations in the extract volume. The larger the volume, the faster the conversion of Ag to AgNPs. This is because a larger extract volume contains a larger number of metabolites; hence, a higher level of synthesis can be expected. These results are consistent with previous research showing that plants contain natural compounds, such as terpenes, alkaloids, flavonoids, phenols, aldehydes, ketones, carboxylic acids, and amides, are excellent reducing and stabilizing agents for nanoparticle synthesis [30].

4 Summary

The presence of phenolic and flavonoid compounds in *M. oleifera* leaf aqueous extract is the primary cause of the reduction of Ag^+ to AgNPs, where the color of the AgNO3 solution slowly changed from pale yellow to dark brown within 24 h. Each synthesis factor in this study clearly shows that further change in conditions increases the yield of AgNPs, in which an increase in pH, extract volume, and temperature facilitates the synthesis of AgNPs using *M. oleifera* leaf extract as a reducing agent. Future works shall determine the physicochemical and morphological properties of the AgNPs using different instruments such as microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, zeta potential etc.

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