Majalah Farmaseutik
ISSN-e: 2614-0064 | ISSN-p: 1410-590x
Volume 22(1) 2026: 84-94

Development of Green Synthesis Silver Nanoparticle with *Ocimum sanctum* Leaf Extract: Characterization and Stability Study

Sukma Uswatun Niswah[1], Nindya Kusumorini[2], Ronny Martien[2*]
[1] Master Student Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Gadjah Mada
[2] Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada
Corresponding author: Ronny Martien | Email: ronnymartien@ugm.ac.id
Submitted: 11-11-2025 *Revised: 12-12-2025* *Accepted: 24-12-2025*

ABSTRACT
This study aimed to synthesize, characterize, and evaluate the stability of silver nanoparticles (AgNPs) produced using *Ocimum sanctum* L. leaf extract through a green synthesis method. The aqueous extract of fresh basil leaves served as a natural reducing and stabilizing agent in the reduction of 1 mM silver nitrate solution at 80 °C, indicated by a color change from light yellow to brown. Phytochemical screening confirmed the presence of phenolic compounds, saponins, and flavonoids responsible for the reduction and stabilization processes. Characterization by UV–Visible spectrophotometry showed a surface plasmon resonance peak at 450 ± 4.93 nm, while particle size analysis indicated a Z-average of 85.77 ± 0.46 nm and a polydispersity index of 0.222 ± 0.003, reflecting uniform and monodisperse nanoparticles. The zeta potential of −14.23 ± 0.30 mV suggested moderate colloidal stability. After 30 days of refrigerated storage, the nanoparticles exhibited a red shift of the absorption peak, an increased particle size of 111.12 ± 1.24 nm, and a slightly higher zeta potential, indicating partial aggregation. These findings demonstrate that *O. sanctum* extract can effectively mediate the eco-friendly synthesis of stable silver nanoparticles, although their long-term stability is influenced by physicochemical interactions of surface biomolecules.
Keywords: green synthesis; *Ocimum sanctum*; silver nanoparticles; stability

INTRODUCTION
Silver (Ag) is a noble metal that has long been utilized for its broad biological activities, including antibacterial, anti-inflammatory, and anticancer effects (Almatroudi, 2020). Compared with other metals, Ag offers advantages such as non-toxicity to human skin and favorable optical properties (Caro *et al*., 2010). In addition, it is widely used as a catalyst, conductor, and stabilizer (Frattini *et al*., 2005; Murugesan *et al*., 2011). The antimicrobial mechanism of silver is mainly attributed to its ability to disrupt bacterial membranes, interfere with metabolism, and inhibit microbial protein synthesis (Haryono *et al*., 2008). Nevertheless, conventional utilization of Ag also faces limitations, particularly in stability and environmental safety during synthesis.

To address these challenges, research has advanced towards improving Ag applications through nanotechnology. Silver nanoparticles (AgNPs) have gained attention for their unique physicochemical properties, including high surface area to volume ratio, enhanced chemical reactivity, and tunable size ranging from 1–100 nm (Ameen *et al*., 2020). These features support their use in wound healing, disinfection, cancer therapy, and antimicrobial treatments (Khan *et al*., 2017). Conventionally, AgNPs are synthesized using chemical or physical methods involving hazardous reducing agents and extreme conditions, making them costly and environmentally unfriendly (Behravan *et al*., 2019). Therefore, recent approaches have focused on alternative synthesis strategies, notably green synthesis, to enhance safety and sustainability.

Green synthesis employs biological systems such as plants, algae, bacteria, and fungi as reducing and stabilizing agents in nanoparticle formation (Behravan *et al*., 2019). This method offers several advantages: it is eco-friendly, economical, and allows better control over particle morphology. Plants, in particular, are widely used due to the presence of phytochemicals like citric acid, flavonoids, and ascorbic acid, which function as natural reducers. Several studies have successfully produced AgNPs using plant extracts such as black pepper (*Piper nigrum*), lemon (*Citrus* *limon*), aloe vera (*Aloe vera*), avocado seed (*Persea americana*), betel leaf (*Piper betle*), and stink bean (*Parkia speciosa*) (Nanda & Zai, 2023). These findings confirm that AgNPs synthesized via green methods not only overcome the drawbacks of chemical synthesis but also enhance biomedical applications.

In this study, basil (*Ocimum sanctum* L.) leaves are selected as a natural reducing agent because they contain alkaloids, saponins, flavonoids, triterpenoids, steroids, and tannins, which provide strong reducing properties through hydroxyl and amine groups (Elemike *et al., 2017*). The aim of this research is to synthesize silver nanoparticles using basil leaf extract and to evaluate their characteristics, physical properties, and stability. The synthesized AgNPs were characterized using UV-Vis spectrophotometry and Particle Size Analyzer (PSA) to determine the size and distribution profile, both immediately after synthesis and following one month of storage to assess their stability, as well as by physical evaluation.

METHODS

Instruments

The instruments used in this study were a mortar and pestle, drop pipette, B-One Digital Ceramic Hotplate (DHS-19C, China), watch glass (Pyrex, USA), chemical spatula, laboratory glassware (Pyrex, Singapore), pH meter (PH009(I)A, China), analytical balance (Mettler Toledo AL204, d = 0.0001 g, Columbus, OH, USA), centrifuge (Mini Spin Plus, Eppendorf AG, Germany), UV-Visible spectrophotometer (Genesys 10 UV Scanning, USA), and Particle Size Analyzer (Zetasizer Nano ZS, Malvern, UK).

Materials

The materials used in this study were silver nitrate ($AgNO_3$) 99.8% (Merck, Darmstadt, Germany), basil leaves (*Ocimum sanctum* L.) obtained from Sleman, Yogyakarta, which were two months old after planting, light green in color, fresh (not wilted), and collected from the first and second nodes, ethanol 96%, $FeCl_3$, NaOH, and HCl (Merck, Darmstadt, Germany), distilled water, aluminum foil, parchment paper, and Whatman filter paper No. 1 with 11 µm pore size.

Procedure

Plant Identification

Ocimum sanctum L. was obtained from Sleman, Yogyakarta. The plant parts collected included flowers, leaves, stems, and roots. Botanical identification was carried out at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

Preparation of *O. sanctum* Aqueous Leaf Extract

Fresh leaves of O. sanctum were thoroughly rinsed with distilled water to remove dust and impurities, followed by distilled water. Approximately 25 g of leaves were finely chopped and homogenized in 100 mL distilled water using a mortar and pestle. The mixture was boiled at 80 °C for 10 min in a 250 mL beaker and then cooled to room temperature (25 °C). The extract was filtered using Whatman filter paper (pore size 1.5 µm) (Singh *et al*., 2018). The filtrate was collected and stored at 4 °C until further use (Rao *et al*., 2013).

Phytochemical Identification of *O. sanctum* Aqueous Leaf Extract

Phenolic Compounds Identification

The identification of phenolic compounds was carried out by mixing 1 mL of *Ocimum sanctum* leaf extract with 2 mL of 96% ethanol and three drops of 2% ferric chloride ($FeCl_3$) solution. The appearance of a reddish-black coloration confirmed the presence of phenolic compounds (Borah and Biswas, 2018; Saranya, Noorjahan, and Siddiqui, 2019).

Saponin Identification

Saponin identification was performed by dissolving 1 mL of *Ocimum sanctum* leaf extract in 1 mL of ethanol and then diluted with distilled water. The mixture was vigorously shaken for a few minutes, and the formation of a stable foam layer of approximately 1 cm indicated the presence of saponins (Isa *et al*., 2023).

Flavonoid Identification

Flavonoid identification was performed by mixing 0.5 mL of *Ocimum sanctum* leaf extract with 2% sodium hydroxide (NaOH) solution, followed by the addition of four drops of 1% hydrochloric acid (HCl). The color change from brown to yellow confirmed the presence of flavonoids (Borah and Biswas, 2018; Saranya, Noorjahan, and Siddiqui, 2019).

Preparation of 1 mM Silver Nitrate Solution

A stock solution was prepared by dissolving 170 mg of silver nitrate 99.8% (Merck, Darmstadt, Germany) in 10 mL distilled water. From this solution, 1 mL was diluted with 100 mL distilled water to obtain a final concentration of 1 mM. The solution was stored in an amber bottle to minimize self-oxidation (Saifuddin *et al*., 2009).

Green Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized by mixing 10 mL of *O. sanctum* aqueous leaf extract with 90 mL of 1 mM $AgNO_3$ solution. The mixture was heated at 80 °C for 15 min. A visible color change from light yellow to brown indicated the formation of AgNPs. The nanoparticles were subsequently collected by centrifugation (Singhal *et al*., 2011).

Characterization of AgNPs

The synthesized nanoparticles were characterized using UV–Vis spectrophotometry and particle size analyzer (PSA). UV–Vis spectrophotometry was employed to monitor the reduction of Ag^+ to Ag^0 by recording the maximum wavelength of the Surface Plasmon Resonance (SPR) band, with spectra scanned in the range of 300–540 nm using ultrapure water as a blank within 24 h of synthesis (Kaviya *et al*., 2011). PSA was used to determine the particle size distribution, including the determination of Z–average particle size, polydispersity index (PDI), attenuator value, and zeta potential in which 1 mL of the AgNP colloid was transferred into a cuvette for measurement (Joshi *et al*., 2012).

Stability Study

The stability study was adapted from Velgosova *et al*. (2017) with several modifications. In this procedure, the AgNP colloid was stored at refrigerator temperature and dark conditions for 30 days. Stability was evaluated through visual observation of color change and instrumental analysis using UV–Vis spectrophotometry and PSA, by comparing the maximum absorption wavelength, particle size distribution, and zeta potential before and after storage.

Data Analysis

Characterization data obtained from UV–Vis and PSA, both before and after one month of storage, were analyzed using IBM SPSS Statistics 27. A paired sample t-test was applied to determine statistically significant differences.

RESULTS AND DISCUSSION

Fresh basil (*Ocimum sanctum* L.) leaves were thoroughly washed with distilled water to remove any adhering dust and impurities. The clean leaves were then finely chopped into small pieces to increase the surface area, allowing more efficient extraction of the active components. The chopped leaves were subsequently ground using a mortar and stamper to facilitate the breakdown of the plant tissue and improve the release of the extract during the heating process as shown in Figure 1.

In this study, the results showed in table 1 that *Ocimum sanctum* (basil) leaves tested positive for the presence of phenolic compounds, saponins, and flavonoids, which is consistent with the findings of Hadipoentyanti and Wahyuni (2008), who reported that basil leaves contain several

Figure 1. Fresh basil (*Ocimum sanctum* L.) leaves and the grinding process

Table I. Phytochemical compounds from the screening test

Chemical Compound	Presence	Description
Phenols	++	Phenolic are present
Saponins	++	Saponin are present
Flavonoids	++	Flavonoid are present

chemical constituents, including essential oils, alkaloids, saponins, flavonoids, triterpenoids, steroids, tannins, and phenols.

The formation of silver nanoparticles through the reduction of silver nitrate using aqueous basil leaf extract can be conveniently monitored by observing the distinct color transition in the reaction mixture. The appearance of a yellowish-brown coloration, as shown in Figure 2, confirms the formation of silver nanoparticles, attributed to the excitation of surface plasmon vibrations. This visual change signifies the reduction of Ag^+ ions to metallic Ag^0 within the nanometer scale (Ramteke *et al*., 2013). Phytochemicals such as proteins, carbohydrates, flavonoids, and phenolic compounds not only serve as capping agents but also act as reducing molecules that facilitate nanoparticle stabilization and control particle size (Vedpriya *et al*., 2010; Collera-Zúñiga *et al*., 2005). The color change occurs due to the phenomenon of surface plasmon resonance (SPR), which arises from the interaction between visible light and metallic nanoparticles possessing a dielectric constant, inducing collective oscillations of surface electrons. The excited surface plasmons in silver nanoparticles are responsible for the resulting color, and particle size further influences the plasmon resonance (Krajczewski *et al*., 2017).

Characterization of AgNPs

Figure 3 illustrates the UV–Vis absorption spectrum of the synthesized silver nanoparticles. The formation of AgNPs was verified by the appearance of a distinct absorption band within the range of 300–540 nm, with a maximum wavelength of 450 ± 4.93 nm, corresponding to the Surface Plasmon Resonance (SPR) phenomenon. This SPR peak arises from the collective oscillation of conduction electrons induced by the reduction of silver ions (Ag^+) to metallic silver (Ag^0), confirming the successful synthesis of stable nanoparticles. The presence and position of the SPR band provide valuable information regarding nanoparticle formation, particle size, and uniformity. These findings

Figure 2. $AgNO_3$ solution (left), basil leaf extract (middle), and AgNP colloid (right)

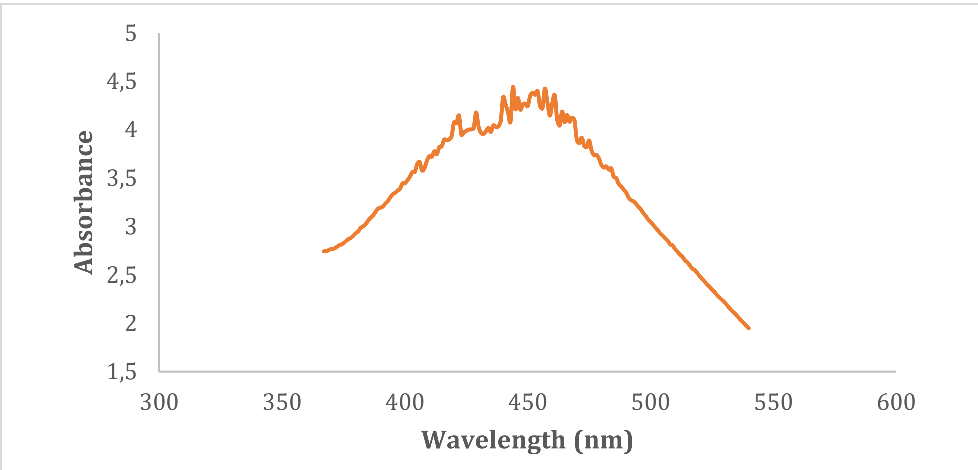


Figure 3. UV–Vis spectrum of silver nanoparticles synthesized using *Ocimum sanctum* leaf extract

are consistent with earlier reports employing Ocimum species as reducing and stabilizing agents in green synthesis approaches.

Qamar *et al*. (2024) reported AgNP formation using *Ocimum basilicum* extract with an SPR peak centered at 344 nm, while Gautam *et al*. (2023) observed a similar band at 452 nm using *Ocimum sanctum* extract. Likewise, Abdelsattar *et al*. (2022) documented AgNP synthesis from *Ocimum basilicum* extract, exhibiting a single well-defined absorption peak between 400 and 430 nm. The comparable results across these studies reinforce that the observed SPR peak in this work is a characteristic Indicator of silver nanoparticle formation.

According to the Particle Size Analyzer (PSA) results, the biosynthesized silver nanoparticles exhibited a Z-average diameter of 85.77±0.46 nm. The Z-average parameter represents the mean hydrodynamic diameter of the particles and serves as the principal indicator of particle size distribution (HORIBA, 2017). The Polydispersity Index (PDI) obtained from the analysis was 0.222±0.003, reflecting the degree of size uniformity within the nanoparticle dispersion. A lower PDI value corresponds to a more uniform particle size distribution (Nurkhasanah *et al*., 2015). Generally, a PDI value close to 1 indicates a broad size distribution and the possible presence of larger particles or aggregates that may lead to sedimentation. Conversely, PDI values below 0.7 are indicative of a narrow size distribution, suggesting uniform and homogeneous nanoparticles (Nurviana *et al*., 2020). Based on this interpretation, the obtained PDI value of 0.222±0.003 confirms that the

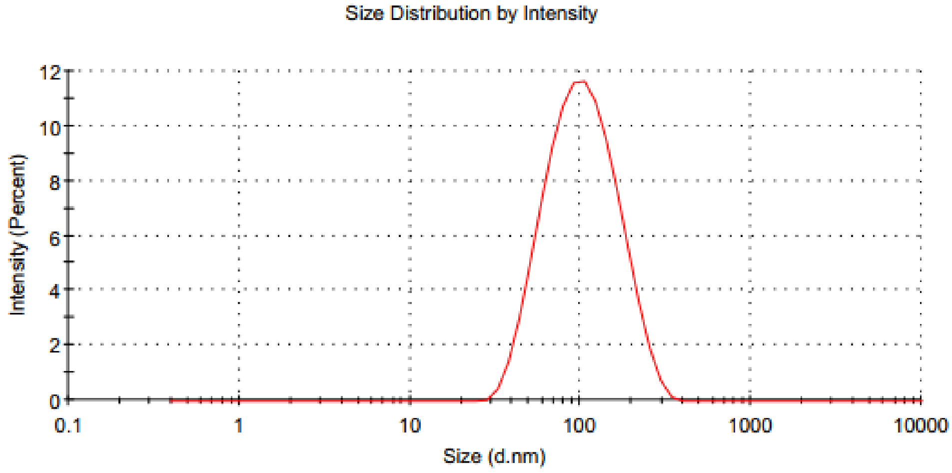


Figure 4. Particle size characterization of silver nanoparticles

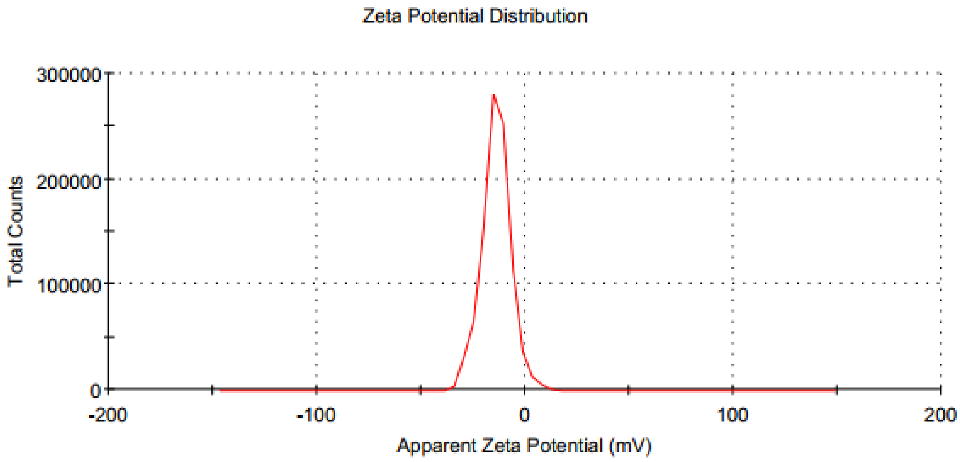


Figure 5. Observation of silver nanoparticle zeta potential

synthesized silver nanoparticles were monodisperse, implying a homogeneous dispersion and stable nanoparticle system (Sreeram *et al*., 2008).

In addition, the analysis also provided an attenuator value, which represents the concentration of particle populations within the sample that may affect the intensity of the incident light. The attenuator automatically regulates the ratio of scattered light intensity to ensure that it remains within a detectable range for the instrument's detector. Excessive particle concentration may result in detector overload due to excessive light scattering (Malvern, 2013). In this study, the obtained attenuator value was 4, indicating that the sample concentration was within the optimal range for accurate measurement.

The results showed that the silver nanoparticle dispersion exhibited an average zeta potential value of −14.23 ± 0.30 mV. Zeta potential serves as an essential parameter for predicting the stability of colloidal systems. The interparticle force interactions play a crucial role in maintaining colloidal stability, which is primarily governed by electrostatic repulsion. When the repulsive forces between particles are reduced, the particles tend to approach one another, leading to aggregation (Pertiwi *et al*., 2019). The negative zeta potential observed for the silver nanoparticles is likely attributed to the capping effect of bioorganic compounds present in the plant extract (Edison and Sethuraman, 2012). Such negative surface charges promote electrostatic repulsion among the nanoparticles, thereby preventing agglomeration and contributing to the formation of a stable colloidal system (Sivaraman *et al*., 2013).

Stability Study

The silver nanoparticle suspension shown in the figure 6 appears to have a dark brown color with a slightly opaque appearance after being stored for one month in the refrigerator. The color remains within the typical range for silver nanoparticles, indicating that the colloidal system has largely maintained its plasmonic characteristics. However, the presence of some sediment or lighter deposits on the beaker wall suggests partial aggregation or sedimentation of nanoparticles over time, which is common during long-term storage. Overall, the brown coloration confirms that the nanoparticles are still present in a dispersed form, though slight instability or particle settling may have occurred.

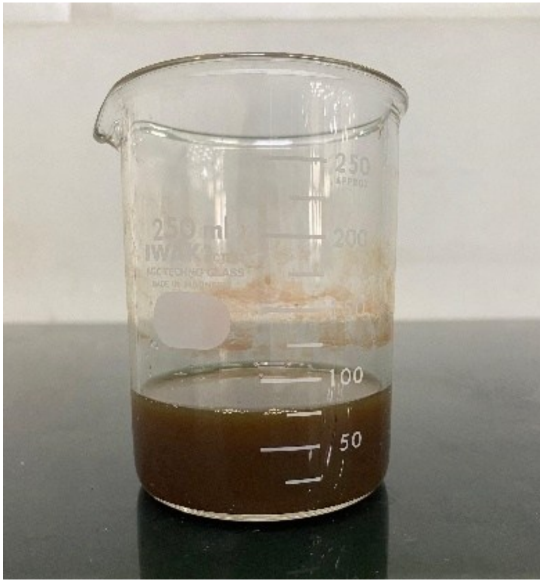


Figure 6. Appearance of silver nanoparticle solution after 30 days of storage in the refrigerator under dark conditions

Similar observations have been reported by AshaRani *et al*. (2008) and Izak-Nau *et al*. (2015), who noted that stored silver nanoparticle suspensions showed changes in color and the appearance of dark precipitates, likely composed of silver oxide (Ag_2O). This phenomenon suggests that prolonged storage may induce surface oxidation and gradual destabilization of the colloidal system.

The UV–Vis spectra of the colloidal silver nanoparticles were measured after storage under refrigeration for one month. A significant change was observed in the spectra of the stored samples compared to freshly prepared ones. The maximum absorption wavelength shifted to 457 ± 3.51 nm, accompanied by a noticeable shoulder at higher wavelengths and a slight loss of symmetry, as shown in Figure 7. This red shift indicates a gradual increase in the mean particle size or partial aggregation over time. However, since no visible aggregation or precipitation was detected, the spectral changes are more likely due to slow nanoparticle growth during storage. These observations align with the findings of Velgosova *et al*. (2024), who reported a similar red shift and spectral broadening in silver nanoparticles synthesized using *Rosmarinus officinalis* extract after 60 days under refrigerated conditions, reflecting size increase without evident agglomeration.

The Z–average value of the silver nanoparticle colloidal solution increased to 111.12 ± 1.24 nm after 30 days of storage under refrigerated and dark conditions, indicating a statistically significant difference (P value < 0.05) compared to the initial measurement. In contrast, Velgosova *et al*. (2024) reported that the mean size of silver nanoparticles synthesized using *Rosmarinus officinalis* extract and stored in the refrigerator for 60 days did not show a significant change, suggesting that particle stability can vary depending on the type of reducing and capping agents present in the plant extract. Furthermore, the increase in particle size after storage may also be associated with the degradation behavior of silver nanoparticles. The spherical structure and larger particle size can enhance the active surface sites and overall surface area, thereby increasing the probability of binding interactions and promoting gradual particle growth or reorganization during storage (Kansal *et al*., 2006; Jaast and Grewal., 2021).

The evaluation of zeta potential provided important insights into the surface charge characteristics and colloidal stability of the biosynthesized silver nanoparticles (AgNPs). According to the findings of Izak-Nau *et al*. (2015), the stability of AgNPs primarily depends on their surface charge, where neutral nanoparticles show minimal change, positively charged particles are the most unstable even when stored in the dark at 4 °C, and the stability of negatively charged particles is strongly influenced by storage conditions. This observation is consistent with the results of the present study. At the initial measurement, the biosynthesized AgNPs exhibited a zeta potential value

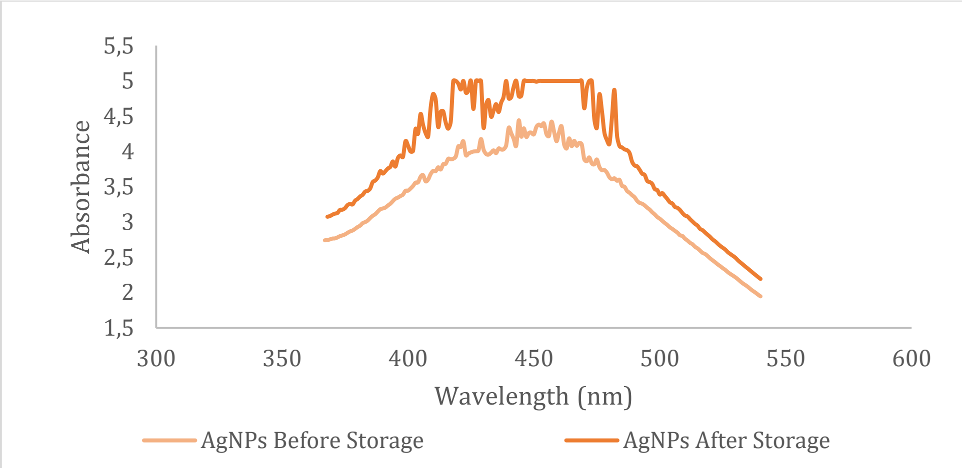
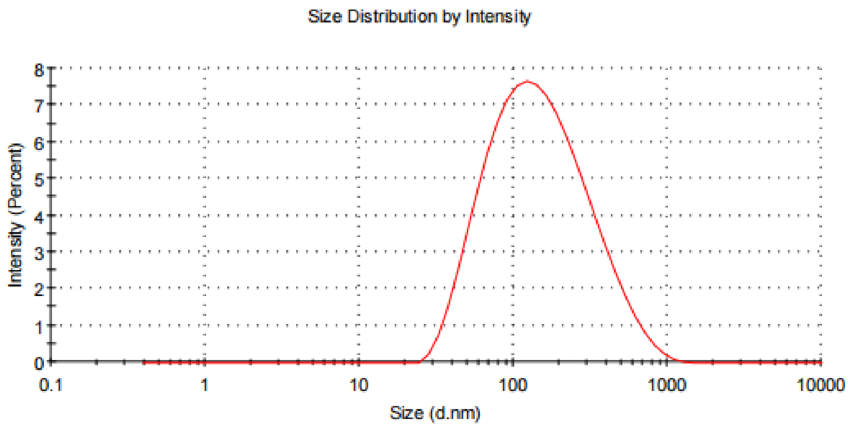
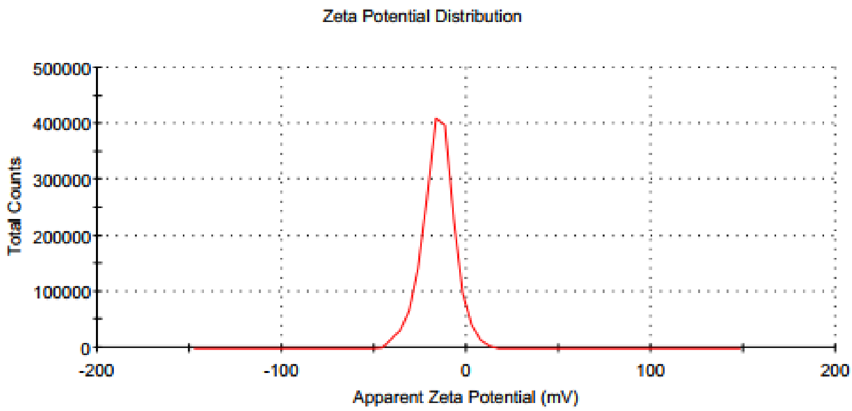


Figure 7. UV–Vis spectra of silver nanoparticles before and after 30 days of storage



(a)



(b)

Figure 8. Particle size (a) and zeta potential (b) of silver nanoparticles after storage

of -14.23 ± 0.30 mV, indicating the presence of negatively charged functional groups derived from bioactive compounds in the plant extract that serve as capping and stabilizing agents.

After 30 days of storage in the refrigerator under dark conditions, the zeta potential slightly increased to -15.53 ± 0.15 mV. The statistically significant increase ($P < 0.05$) suggests stronger electrostatic repulsion between nanoparticles, confirming that a higher density of negatively charged groups contributes to improved colloidal stability of AgNPs in aqueous medium. In the case of negatively charged silver nanoparticles, the capping molecules are relatively small, forming a very thin surface layer, which may accelerate the dissolution process and consequently contribute to the increase in particle size during storage (Izak-Nau *et al*., 2015).

CONCLUSION

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Ocimum sanctum* leaf extract as a natural reducing and stabilizing agent. Characterization confirmed the formation of monodisperse, negatively charged AgNPs with a distinct SPR band. After 30 days of refrigerated storage under dark conditions, all measured parameters, including SPR wavelength, particle size, and zeta potential, showed statistically significant differences, indicating structural modification and partial instability over time. These findings suggest that while *O. sanctum* effectively facilitates AgNP synthesis, long-term stability remains influenced by the physicochemical interactions of the capping biomolecules during storage.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Pharmaceutics Laboratory, Faculty of Pharmacy, Universitas Gadjah Mada, for providing the necessary facilities and support for this research.

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