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Green Synthesis of Silver Nanoparticles from Soursop Leaf Extract (Annona Muricata Linn.)

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A B S T R A C T

The green synthesis of silver nanoparticles using plant extracts has attracted considerable attention in recent years. In this work, silver nanoparticles were produced employing soursop (Annona muricata Linn) leaf extract as a natural bioreductant. The study examined the effect of $AgNO_3$ concentration, followed by optimization of the ratio between the bioreductant and $AgNO_3$ solution to determine the most stable nanoparticle formation. Particle size analysis (PSA), scanning electron microscopy (SEM), and UV-Vis spectrophotometry were used to characterize the nanoparticles. With a bioreductant volume double that of the silver nitrate solution, ideal conditions were reached at 1 mM $AgNO_3$. PSA findings indicated a size variety from 40 to 170 nm with an average size of 170.6 nm, whilst SEM pictures showed mostly spherical particles with somewhat low homogeneity. All things considered, this green synthesis method provides an economical, environmentally responsible, and sustainable way to produce silver nanoparticles

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INTRODUCTION

At present, many researchers are focusing on innovations in nanotechnology. Nanotechnology refers to techniques for designing and utilizing materials with nanoscale structures. Among its various applications, silver nanoparticles have received significant attention, and one common approach for their synthesis is chemical reduction. This approach is appropriate for large-scale production, somewhat easy to use, and economical (Kudle et al., 2013).

Due to its environmentally benign, economical, and sustainable production method, silver nanoparticles (AgNPs) made from plant extracts have been widely produced in recent years.These biogenic nanoparticles demonstrate remarkable versatility and are increasingly applied across diverse fields, including photocatalysis for the degradation of organic pollutants, environmental remediation for mitigating hazardous contaminants, and biomedical applications such as antimicrobial, anticancer, wound healing, and targeted drug delivery systems (Shahzadi et al., 2025; Akhtar et al., 2025).

However, although effective in producing stable colloidal nanoparticles and preventing agglomeration, the use of chemical reducing agents often generates hazardous by-products that may harm the environment. To address this issue, biosynthesis, also known as green synthesis, has been explored as an alternative method because it employs safer, eco-friendly reducing agents (Sari et al., 2017).

The growing interest in plant-based green synthesis is further supported by recent studies that highlight its ability to generate stable, size-controlled, and biologically active nanoparticles while minimizing toxic by-products, making this approach highly attractive for both environmental and biomedical applications (Tiwari, et al., 2024; Yadav, et al., 2025).

Plant extracts serve as natural bioreductants, capable of reducing Ag^{+} ions to Ag^{0}, thereby forming silver nanoparticles. Extracts rich in According to Sari et al. (2017), antioxidant substances including vitamin C and flavonoids work especially well as reducing agents. With secondary metabolites such as steroids, flavonoids, coumarins, alkaloids, and tannins, soursop (Annona muricata Linn.) leaf extract is one intriguing option (Adri et al., 2013). It is thought that these bioactive substances contribute to the reduction process that results in the production of nanoparticles.

Based on this background, the present study aims to synthesize silver nanoparticles using soursop leaf extract as a bioreductant and to evaluate their characteristics through UV–Vis spectrophotometry and particle size analysis.

LITERATURE REVIEW

Green Synthesis of Silver Nanoparticles from Soursop Leaf

The ecologically benign, economical, and chemical-free green production of silver nanoparticles from leaf extract has been the subject of much research. Typically, the manufacturing process uses bioactive chemicals found in the soursop leaf extract to reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}). The green synthesis process is a sustainable and biocompatible method of producing nanoparticles since it stays away from harmful chemicals and unfavorable physical conditions (Gavamukulya et al., 2019).

Mechanism of Green Synthesis of Silver Nanoparticles

In order to transform silver ions (Ag^{+}) into metallic silver nanoparticles (Ag^{0}), the green synthesis of silver nanoparticles (AgNPs) uses plant extracts, microbes, or other biological components as reducing and stabilizing agents. The method usually consists of the following phases when employing plant extracts, including those from soursop leaves (Iravani, 2011; Mittal et al., 2013):

Reduction of Silver Ions, the bioactive compounds present in the plant extract—such as flavonoids, phenolics, alkaloids, terpenoids, and proteins—serve as organic reducing agents. These substances reduce the silver ions (Ag^{+}) in the solution to elemental silver (Ag^{0}) by donating electrons to them. The nucleation process, in which tiny silver atoms begin to group together, is started by this reduction.

Nucleation and Growth, once silver atoms form, they begin to nucleate and aggregate into small clusters or seeds. These nuclei then grow through further reduction and agglomeration, forming nanoparticles. The growth phase determines the final size and shape of the nanoparticles.

Stabilization and capping simultaneously, other bioactive molecules in the extract bind to the surface of the growing nanoparticles, acting as capping agents. These molecules stabilize the nanoparticles by preventing excessive aggregation, controlling their size distribution, and providing colloidal stability. The capping also protects the nanoparticles from oxidation and helps maintain their dispersity in the solution. After sufficient nucleation, growth, and stabilization, stable silver nanoparticles are formed in the colloidal solution.

Synthesis and Characteristics of Silver Nanoparticles

Various studies have reported the synthesis of silver nanoparticles (AgNPs) using Soursop leaves are dried, ground, and extracted using solvents such as water or ethanol. The extract is then mixed with a silver nitrate ($AgNO_3$) solution and incubated at room temperature or a specific temperature to allow the reduction of silver ions into AgNPs. Characteristic of the silver nanoparticle could be interpreted from size, crystal structure and morphology. The nanoparticles show good stability under different pH, temperature, and storage conditions, with zeta potential measurements indicating a negative charge that supports colloidal stability (Gavamukulya et al., 2019).

METHODOLOGY

Equipment and Materials

The chemicals are Polyvinylpyrrolidone (PVP), Dragendorff's reagent, sulfuric acid (H_2SO_4), acetic anhydride, hydrochloric acid (HCl), magnesium powder, ferric chloride ($FeCl_3$), silver nitrate ($AgNO_3$), and methanol (CH_3OH) were all purchased from Merck. Soursop leaves were collected from Lempake Subdistrict, North Samarinda District, East Kalimantan, Indonesia. The tools used in this research include beaker, volumetric flask, volume pipette, bulb, dropper pipette, rotary evaporator, hot plate, magnetic stirrer, analytical balance, UV-Vis spectrophotometer, transmission and scanning electron microscopes, and particle size analyzer.

Sample preparation and Extraction of Soursop leaf

Fresh green leaves were carefully selected for this study, and twigs from the tree were also collected to facilitate plant identification. All experimental procedures were conducted at the Laboratory of Plant Anatomy and Systematics. A total of 500 g of soursop leaves were obtained, washed, air-dried for 14 days, and subsequently cut into small pieces measuring approximately 0.5–1 cm. The extraction of soursop leaves was performed using the maceration technique following the method described by Az-Zahra et al. (2020). Specifically, 200 g of leaves were macerated in 4 L of methanol for seven days. The resulting mixture was filtered to obtain the methanolic extract (filtrate) and the leaf residue, which appeared brown in color. A rotary evaporator was then used to concentrate the filtrate. Alkaloids, flavonoids, phenolics, steroids, and saponins were detected by phytochemical screening of the soursop leaf extract.

Phytochemical Tests

Alkaloid Test

One milliliter of soursop (Annona muricata Linn.) leaf extract was treated with two drops of Dragendorff's reagent. An orange-brown precipitate formed, indicating a positive alkaloids result (Puspitasari, 2018).

Steroid and Triterpenoid Test

Two drops of acetic anhydride were added to the extract, and then two drops of concentrated H_2SO_4. The creation of a brown or violet ring signified a positive triterpenoid test, whereas the presence of a bluish-green ring indicated a positive steroid test. (Ciulei, 1984).

Flavonoid Test

One milliliter of After combining the extract with 0.5 mL of concentrated HCl, 1 mg of magnesium powder and 1 mL of amyl alcohol were added. A color shift to red or orange signified a positive flavonoid test. (Puspitasari, 2018).

Phenolic Test

One milliliter of extract was treated with five drops of 5% $FeCl_3$ solution and vigorously shaken. A positive test for phenolics was indicated by a color change to green, purple, red, black, or blue (Harborne, 1987).

Saponin Test

One milliliter of extract was mixed with 2 mL methanol and shaken vigorously for 10 seconds. A positive saponin test was indicated by the formation of a stable froth (Puspitasari, 2018).

Synthesis of Silver Nanoparticle

AgNO3 1 mM and soursop leaf extract 0.2% (w/w) as bioreductant was added to a beaker glass in accordance with Table 1, followed by the addition of 0.20 mL of 17% PVP. Additionally, stirred for two hours at room temperature until the color turned brown, indicating the formation of nanoparticles. A UV-Vis spectrophotometer was then used to characterize the solution and assess its stability.

Table 1. Ratio of Bioreductor Volume with $AgNO_3$ Solution

No.	$AgNO_3$:Bioreductant	Bioreductant (mL)	$AgNO_3$ 1 mM (mL)
1	2:1	20	10
2	1:1	15	15
3	1:2	10	20
4	1:3	10	30

Stability of Silver Nanoparticles Through Several Analysis of Characterization

The produced silver nanoparticles were evaluated using a UV–Vis spectrophotometer to validate their production and evaluate their stability. By evaluating the spectra of nanoparticle solutions made with various ratios of soursop leaf extract as the bioreductant and varied concentrations of $AgNO_3$, the highest absorption wavelength was identified. Colloidal silver nanoparticles' distinctive absorption peaks were seen in the range between 370 and 650 nm. In order to evaluate the stability of the nanoparticles, absorbance measurements were tracked for seven days. Particle size analysis (PSA), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) were then used to further evaluate the most stable nanoparticles. With typical micrographs displaying the structural characteristics of the produced nanoparticles, these sophisticated characterizations provide comprehensive information on particle size distribution and surface morphology.

RESULTS AND DISCUSSION

A total of 287 grams of soursop leaves (*Annona muricata Linn*.) that had been previously prepared were extracted using methanol as the solvent. The extract was then concentrated, yielding 23.1 grams of thick soursop leaf extract, resulting in a percentage yield of 8.0487%. Subsequently, a phytochemical screening was conducted.

Phytochemical screening is a method used to analyses the presence and types of active chemical compounds found in plants (Illing et al., 2017). This test was carried out to identify the secondary metabolite compounds present in the concentrated soursop leaf extract. The results of the phytochemical screening are presented in Table 2.

Table 2. Secondary Metabolite Compounds in Soursop Leaves (*Annona muricata* Linn.)

Type of Compound	**Methanol Extract of Soursop Leaves**
Alkaloids	+
Steroids	-
Triterpenoids	+
Phenolics	+
Flavonoids	+
Saponins	-

Note:
(+) = presence of secondary metabolite compound
(-) = absence of secondary metabolite compound

According to Table 2, the secondary metabolites present in *Annona muricata* Linn. leaf extract are alkaloids, triterpenoids, phenolic, and flavonoids. By reacting AgNO3 at a concentration of 1 mM with a bioreductant of soursop leaf extract at a concentration of 0.2% (W/W) that contains flavonoid compounds that can reduce metals, silver nanoparticles were created using a soursop leaf extract bioreductant (Maryani et al., 2017). ¬ In order to optimize the production of silver nanoparticles, the volume ratio between the bioreductant and $AgNO_3$ solution was changed.

A shift in the $AgNO_3$ solution's hue from clear to yellowish-brown indicates the creation of silver nanoparticles. Figure 1 displays the final produced silver nanoparticles.

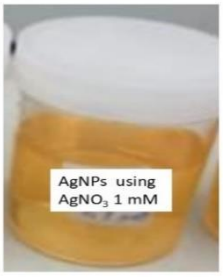

Figure 1. Silver nanoparticles (AgNPs) synthesized using 1 mM $AgNO_3$ solution with soursop (*Annona muricata* Linn.) leaf extract as a bioreducing agent.

The synthesis of silver nanoparticles was conducted by varying the volume ratios of the bioreductor to $AgNO_3$ at 2:1, 1:1, 1:2, and 3:3. The stability of the synthesized nanoparticles was subsequently evaluated over a period of 7 days using UV-Vis spectrophotometry, as illustrated in Figure 2.

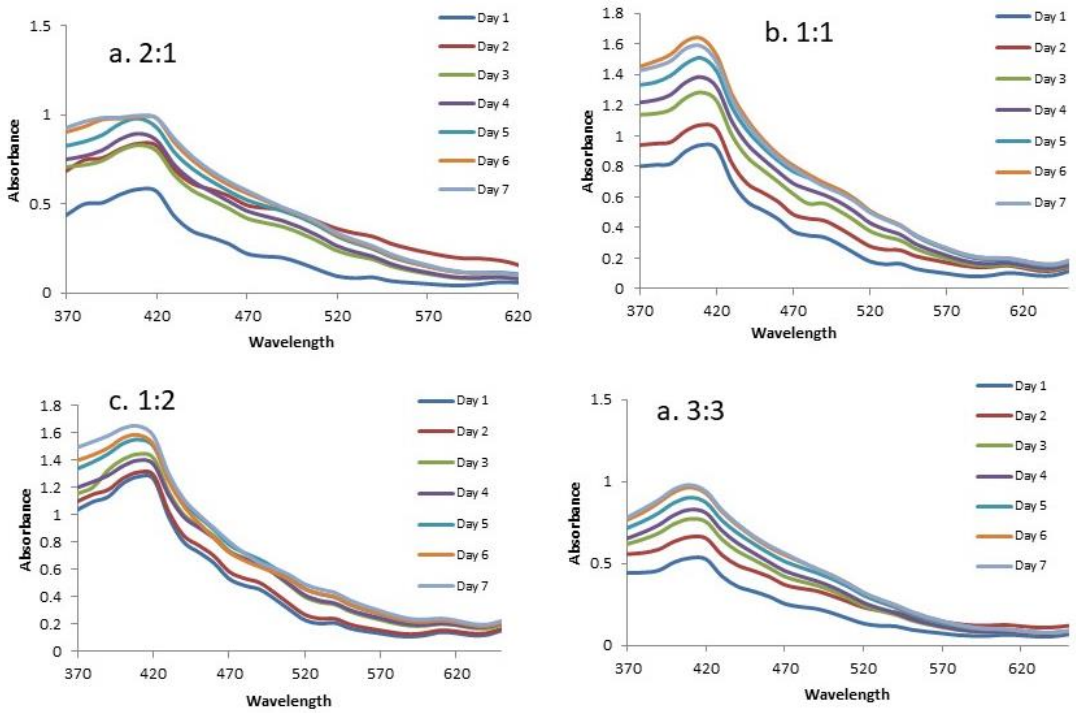

Figure 2. The results of observations on the stability of silver nanoparticles with bioreductors for 7 days on volume ratio of $AgNO_3$: Bioreductant (a) 2:1 , (b) 1:1, (c) 1:2 and (d) 3:3

After obtaining a yellowish-brown solution, The stability of the produced silver nanoparticles was then assessed after showing the creation of silver nanoparticles. As seen in Figure 4.4, the maximum absorbance value was displayed by silver nanoparticles with a 2:1 volume ratio. It is evident from the study that a greater absorbance value is correlated with a larger volume of the bioreductant. The reason for this phenomena is that more bioreductant causes more Ag^+ ions to be reduced to Ag^0 ions, which in turn produces more silver nanoparticles (Haryani et al., 2016).

Based on the stability and optimization studies of silver nanoparticles synthesized using soursop (*Annona muricata* Linn) leaf extract as a bioreductant, the most stable formulation with the highest nanoparticle yield was achieved at a silver nitrate ($AgNO_3$) concentration of 1 mM, using a bioreductant volume twice that of the $AgNO_3$ solution. This optimized nanoparticle suspension will subsequently be characterized using a Particle Size Analyzer (PSA) to determine the particle size distribution of the synthesized silver nanoparticles.

The result from the characterization of silver nanoparticles using particle size analyzer (PSA) shown in figure 3.

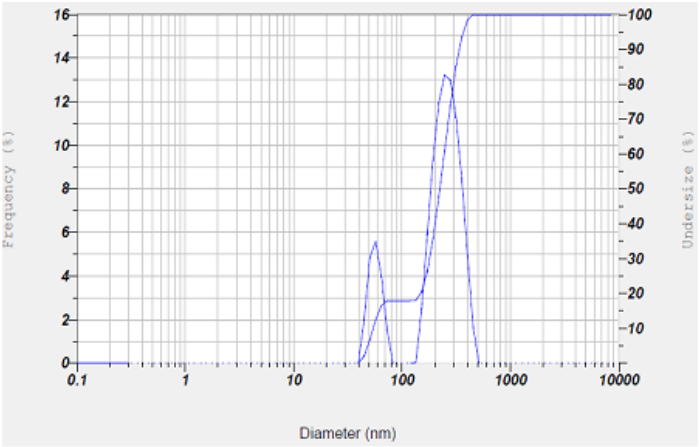

Figure 3. Particle Size Analyzer (PSA) characterization results on silver nanoparticles

The size of the silver nanoparticles ranged from 40 to 170 nm, according to the PSA test findings.

In this work, further characterisation was carried out using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to examine the morphological and structural characteristics of the silver nanoparticles. Figure 4 displayed the silver nanoparticles' surface morphology.

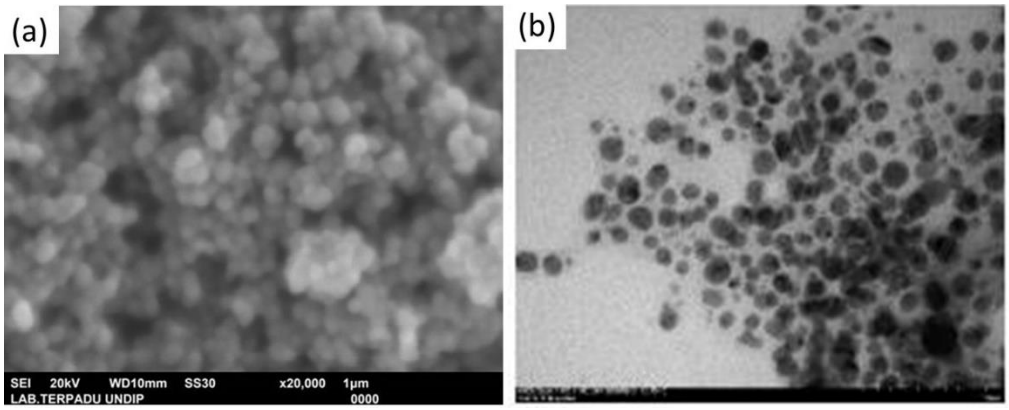

Figure 4. Surface morphology of silver nanoparticles synthesized using a bioreductant from soursop (*Annona muricata* Linn) leaf extract: (a) observed using SEM, and (b) observed using TEM.

CONCLUSION AND RECOMMENDATION

The secondary metabolites of soursop leaves (Annona Muricata Linn.) is an alkaloid, triterpenoid, phenolic and flavonoid. Alkaloids are secondary metabolites characterized by cyclic structures containing nitrogen atoms with a negative oxidation state. The presence of alkaloids can be tested using Dragendorff's reagent, which gives a positive result in the form of an orange-brown precipitate or solution (Puspitasari, 2018). Based on the data, the soursop leaf extract tested with Dragendorff's reagent produced an orange-brown solution, indicating the presence of alkaloid compounds as secondary metabolites.

Steroids are triterpenoid compounds that have a cyclopentanoperhydrophenanthrene ring as their basic structure. Steroids and triterpenoids can be tested using the Liebermann-Burchard reagent, a mixture of glacial acetic acid and concentrated sulfuric acid. The production of a bluish-green ring indicates a good result for steroids, whereas the formation of a brown or violet ring indicates a positive result for triterpenoids (Ciulei, 1984). According to the findings, the soursop leaf extract only produced a brown ring when examined with the Liebermann-Burchard reagent, suggesting the presence of triterpenoids and the lack of steroid chemicals.

Flavonoids are polyphenolic compounds with a structure derived from the anti-aromatic flavan or 2-phenylbenzopyran. Flavonoids can be identified using a test involving concentrated HCl and magnesium powder, which yields a red or orange colour as a positive result (Puspitasari, 2018). The test results showed that the soursop leaf extract produced a yellow colour when reacted with concentrated HCl and magnesium powder, indicating the presence of flavonoid compounds.

Phenolics are compounds containing aromatic rings substituted with one or more hydroxyl groups, typically derived from the shikimic acid and phenylpropanoid metabolic pathways. Phenolic compounds can be detected using $FeCl_3$ reagent, which gives a positive reaction characterized by the formation of a green, purple, red, black, or blue-coloured solution (Harborne, 1987). In this study, the reaction yielded a dark green solution, confirming the presence of phenolic compounds in the soursop leaf extract.

Saponins are glycosidic compounds consisting of a triterpenoid or steroid aglycone linked to one or more sugar moieties. Saponins can be tested by adding methanol and vigorously shaking the solution for 10 seconds. A positive result is indicated by the formation of a stable foam on the surface (Puspitasari, 2018). In this study, no stable foam was observed, suggesting that the soursop leaf extract does not contain saponin compounds.

Phytochemical screening confirmed that soursop (Annona muricata Linn.) leaf extract contains flavonoid compounds. Flavonoids act as effective bioreductants for silver nanoparticle synthesis because their redox potential is lower than that of Ag^+ ions, enabling the reduction of Ag^+ to Ag^0 (Maryani et al., 2017).

The stability of silver nanoparticles synthesized with varying ratios of bioreductant to $AgNO_3$ was evaluated by monitoring their absorbance spectra (Figure 2). Nanoparticles prepared with volume ratios of 2:1, 1:1, 1:2, and 3:1 consistently exhibited maximum absorbance peaks at ~410 nm, which remained

stable from day 1 to day 7. These findings indicate that the variations in the bioreductant-to-$AgNO_3$ ratio did not cause significant shifts in the surface plasmon resonance wavelength, demonstrating the overall stability of the nanoparticles (Taba et al., 2019).

Among the tested ratios, the 1:2 variation yielded the highest absorbance intensity (Figure 2). This trend suggests that increasing the volume of bioreductant enhances nanoparticle formation, as a greater availability of reducing agents promotes more extensive conversion of Ag^+ ions into Ag^0, thereby increasing nanoparticle yield (Maryani et al., 2017).

Stability testing and optimization of silver nanoparticles synthesized using soursop leaf extract (Annona muricata Linn.) as a bioreducing agent demonstrated that the most stable nanoparticles with the highest yield were obtained at an $AgNO_3$ concentration of 1 mM, with the bioreductor volume maintained at twice that of $AgNO_3$. These nanoparticles were subsequently characterized using a Particle Size Analyzer (PSA) to determine their particle size, expressed as the radius of spherical particles. As shown in Figure 3, the characterization results indicated that the nanoparticles ranged from 40 to 170 nm, with an average size of 170.6 nm. The observed variation in particle distribution was attributed to agglomeration occurring during the synthesis process (Masakke et al., 2014).

Further Using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), the silver nanoparticles made from Annona muricata Linn. leaf extract were characterized in order to assess their morphology, shape, and size distribution. SEM and TEM examinations verified that the nanoparticles had a highly uniform size distribution and a mostly spherical shape, as shown in Figure 4. There was no discernible trace of agglomeration, and the particles were evenly distributed.

FUTHER STUDY

Future Research Directions on Green Synthesis of Silver Nanoparticles from Soursop Leaf are optimization of synthesis parameters future studies can focus on optimizing synthesis conditions such as pH, temperature, reaction time, and concentration of silver nitrate and soursop leaf extract. Fine-tuning these parameters can improve nanoparticle yield, control particle size distribution, and enhance stability for specific applications. Furthermore, extensive in vitro and in vivo studies are needed to evaluate the biomedical potential of soursop-mediated AgNPs, including antimicrobial, anticancer, and anti-inflammatory effects. Toxicity and biocompatibility assessments should be carried out to ensure safety for clinical or pharmaceutical use.

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