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Green Synthesis of Silver Nanoparticles with Snake Fruit Peel Extract: A Preliminary Study For Optimization of The Preparation Technique

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Abstract: In Indonesia, the pulp of snake fruit is consumed while the peel remains unused. In this context, the phytochemical content of snake fruit aqueous extract was used as a reducing and capping agent in the preparation of silver nanoparticles (AgNPs). According to the phytochemical screening, snake fruit peel containing alkaloids, flavonoids, tannins, saponins, and polyphenols was used to synthesize AgNPs. Therefore, this research aimed to optimize the synthesis of AgNPs using snake fruit peel extract (*Salacca zalacca*) in terms of synthesis time, temperature and phytochemical screening. AgNPs were successfully synthesized using a volume ratio of 2:15 (20 mL of 1.00 mM $AgNO_3$ to 150 mL of snake fruit peel extract) in a sealed container in a dark room. Furthermore, characterization was carried out using a UV-Vis spectrum and Fourier Transform Infrared Spectroscopy (FTIR). The UV-Vis spectrum characterization in a solution incubated for 30 minutes at 30 °C with a concentration of 1.00 mM $AgNO_3$ was differentiated at a wavelength of 410 nm with an absorbance of 2.361. According to the FTIR characterization, there was an increase in the intensity of the O – H functional groups in the AgNPs solution compared to snake fruit peel extract. The results showed that the synthesis of AgNPs from snake fruit peel extract was optimal at a concentration of 1.00 mM $AgNO_3$ at 30 °C and 30 minutes of incubation.

Keywords: AgNPs; Snake Fruit Peel; Phytochemical; Green Synthesis; Optimization

Introduction

In the world, nanoscience and nanotechnology research is advancing rapidly. Nanotechnology contributes to almost every field of science, including physics, materials science, chemistry, biology, computer science, engineering, and healthcare (Bayda et al., 2020). Various methods to synthesize silver nanoparticles (AgNPs) have been reported. Physical and chemical synthesis are well known but include hazardous materials and require high temperatures with adverse effects on the environment (Yousaf et al., 2020). Recently, biological methods have been selected as an alternative in the synthesis of nanoparticles using plants and microorganisms (Abdel-Halim et al., 2011; Iravani et al., 2014; Makarov et al., 2014). Some examples of plants reported in the synthesis are galangal(Rizkita et al., 2023), sambiloto (Purnomo et al., 2017), and snake fruit (Prodjosantoso et al., 2019).

Nanoparticle synthesis by biosynthesis method is a form of application of green chemistry principles. This principle pertains to sustainable chemical processes, conservation of energy, the utilization and production of chemicals that are safer and more environmentally

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friendly for human health, reducing the risks associated with global warming, as well as the management of natural resources and agricultural waste (Kharissova et al., 2019). In addition, the biosynthesis method can include microorganisms and plant extracts (Chung et al., 2016; Iravani, 2011; Iravani et al., 2014), which are known to be fast in the reaction process (Yousaf et al., 2020).

Previous research was carried out using fruit waste as a reductor agent in the synthesis of AgNPs. Grape and orange peel extracts containing phenolic compounds have been reported to be reductants (Soto et al., 2019). This research showed that the successfully synthesized AgNPs had a spherical shape with a size of 10-50 nm and also reported antibacterial activity against gram-negative bacteria *Escherichia coli*. Another research using citrus fruit peels (*Citrus limon*, *Citrus sinensis*, and *Citrus limetta*) suggested that the synthesis could be performed only with a one-step protocol. This was because the resulting AgNPs had antimicrobial activity against gram-negative *E. coli* and gram-positive *Staphylococcus aureus*. Furthermore, Annu et al. (2018) reported that the AgNPs produced had good antioxidant activity.

Biodiversity is important in the development of silver nanoparticles with green synthesis methods, namely biosynthesis. In this context, snake fruit (*Salacca Zalacca*) has been reported as a good fruit for biosynthesis of AgNPs. Furthermore, research on snake fruit includes the production of bioethanol by fermentation (Fatimah et al., 2013), antimicrobial tests against *E. coli* (Nurnia et al., 2014), and antioxidant activity (Ariviani & Parnanto, 2013) but the peel has not been analyzed as a bioreductor for AgNPs. The results of phytochemical tests of snake fruit peel containing flavonoids, tannins and alkaloids have been reported (Putri, 2016). According to Gladyshev et al. (2013), the ethanol extract of snake fruit peel contained secondary metabolites namely alkaloids, polyphenols, flavonoids, tannins, quinones, monoterpenes and sesquiterpenes with a moisture content of 13.25%, total ash content of 5.61%, and acid insoluble ash content of 0.50%. Based on several analyses, snake fruit peel needs to be screened by phytochemical compounds and investigated to become a reductant and stabilizer in the synthesis of AgNPs.

Method

Materials

The materials used in this method were snake fruit peel, distilled water, $AgNO_3$ solution (Merck) (0.25 mM, 1.00 mM, 2.00 mM, and 4.00 mM), and colloidal silver nanoparticles (Maxlab). Meanwhile, the tools included drying cabinet, a set of glassware, filter paper, buchner filter, aluminium foil, UV-Vis spectrophotometer PG Instruments T60, measuring pipette, mortar, sieve, a magnetic stirrer, hot plate, pH meter, and brown bottle.

Methods

Sample Preparation

The snake fruit peel was dried using a drying cabinet at 50 °C for 72 hours before grinding and sieving with a size of 20 mesh. Furthermore, 100 grams of the mashed peel was mixed with 400 mL of distilled water and macerated with stirring for 60 minutes followed by filtering using a Buchner filter. The filtrate obtained was measured for pH before being used as a reductant for the manufacture of AgNPs.

Phytochemical Screening (Shabir et al., 2018)

Alkaloid Identification

A total of 2 mL of extract solution was added with 5 mL of 2N HCl and the solution obtained was divided into 3 test tubes. A total of 3 drops of the first, second and third tubes were added to Wagner, Dragendroff, and Mayer reagents, respectively. The formation of an orange-to-red-brown, orange, and white-to-yellowish precipitate in the first, second, and third tubes reported the presence of alkaloids.

Flavonoid Identification

Approximately 2 mL of extract solution was heated to boiling for 5 minutes. Subsequently, a small quantity of Mg powder and 1 mL of concentrated HCl were added to the solution and agitated to obtain a positive result of red, yellow, or orange colors.

Identification of Saponins

Saponins were detected by foam test in hot water and stable foam was reported for 5 minutes without disappearing on the addition of 1 drop of HCl 2 N.

Identification of Steroid and Terpenoid

A total of 2 mL of extract solution was added with 10 and 2 drops of glacial CH_3COOH and concentrated H_2SO_4, respectively. The solution was shaken gently and left for a few minutes. Steroids obtained a blue or green color, while triterpenoids produced a red or purple color.

Identification of Tannins

A total of 2 mL of extract solution was added to 3-5 drops of 1% Iron (III) Chloride solution. The changes observed included the formation of a dark blue or greenish-black color, reporting the presence of tannin compounds.

Identification of Polyphenol (Mentari et al., 2018)

Approximately 2 ml of extract solution was put into a test tube and 2-5 drops of 5% $FeCl_3$ solution was added. Subsequently, the formation of a blue-black color showed the presence of phenolic compounds.

Preparation of Snake fruit Peel Extract (SPE) AgNPs (Prodjosantoso et al., 2019)

A total of 150 mL of the extract was added sequentially with $AgNO_3$ solution (0.25 mM, 1.00 mM, 2.00 mM, and 4.00 mM) dropwise until the color turned more intense in a water bath at 30 ^{0}C, 50 ^{0}C, and 75 ^{0}C and incubated for 15, 30, 60, 120, 240, and 1440 minutes before covering with aluminium foil.

Analysis of AgNPs

Spectrophotometer UV-Vis

The solution was measured using a UV-Vis spectrophotometer with a wavelength of 300 - 700 nm to determine the peak absorbance of the absorption wavelength of the nanoparticles formed.

Spectrophotometer FTIR

Characterization of functional groups in the samples was carried out using an FTIR spectrophotometer. Measurement of AgNPs samples used FTIR PerkinElmer Spectrum IR 10.7.2 while salak peel and $AgNO_3$ adopted FTIR Shimadzu. Meanwhile, the condition of the samples tested with FTIR was the form of solids.

Result and Discussion

Phytochemical Screening of SPE

Phytochemical screening test of SPE is observed from the color change of the sample given reagents. Based on the results, salak peels contain alkaloid, flavonoid, saponin, polyphenol, and tannin compounds as reported in Table 1.

Table 1. Phytochemical Screening Results of SPE

Compound	Reagent	Color	Results
Alkaloid	a. Wagner	Orange	+
	b. Dragendroff	Orange	+
	c. Mayer	Yellowish	+
Flavonoid	Mg and HCl	Orange	+
Steroid and terpenoid	CH_3COOH glacial and H_2SO_4	Orange	-
Tannin	$FeCl_3$	Green-blackish	+
Saponin	Foam test	Stable foam for 5 minutes	+
Polifenol	$FeCl_3$	Blue-blackish	+

Description: (+) = Color Change Occurs, (-) = No Color Change Occurs

Synthesis Optimization of Silver Nanoparticles from SPE

This research followed the procedure reported by (Prodjosantoso et al., 2019) with modifications and AgNPs were synthesized using a volume ratio of 2:15 (20 mL) $AgNO_3$ to 150 mL SPE as reducing and capping agents. In this context, the incubation procedure was carried out in a closed container in a dark room.

The synthesis of AgNPs can be described in three stages. The first stage, known as the reduction phase is when biomolecules or secondary metabolites reduce silver ions (Ag^+) to metal (Ag^0). The second stage is called the nucleation phase which occurs when the metal plate (Ag^0) tends to migrate towards other particles, forming agglomerates or clusters. The third stage is known as the growth phase, which is when small adjacent nanoparticles start to coalesce to form larger particles (Zulaicha et al., 2021). This spontaneous process is thermodynamically controlled, where larger particles are energetically favored and this is known as Ostwald maturation (Makarov et al., 2014). The growth stage ends with the formation of nanoparticle size and shape, which will be determined by the synthesis conditions such as temperature, $AgNO_3$ concentration, and synthesis time.

The formation of AgNPs in SPE can be observed from the color changes and UV-Vis spectrophotometer absorbance measurements with a wavelength range of 350 - 450 nm (Fu et al., 2021). During incubation in a dark room, silver ions are subjected to a reduction reaction of silver ions (Ag^+) for a certain period to produce AgNPs (Ag^0) (Thongnopkun et al., 2018). The color change in the research is from brown to dark brown as reported in Figure 1 and this is caused by the excitation of surface plasmon vibrations on AgNPs (Rizkita et al., 2023). Meanwhile, the color change in AgNPs solution depends on the incubation time. The longer the incubation time, the more intense the color produced due to the reduction of silver ions (Ag^+) into nanoparticles (Ag^0) (Fu et al., 2021).

Figure 1: $AgNO_3$ solution (a), SPE (b), AgNPs solution produced (c).

Phytochemicals and secondary metabolites in plants are considered as reducing agents in green synthesis. Even though complete information on the

synthesis of metal nanoparticles using plants has not been well understood, plant secondary metabolites can lead to the production of metal nanoparticles. Oxygen generated from the degraded secondary metabolites connects the reduced metal ions. In Figure 2, saponin glycones in plant extracts interact with Ag^+ ions through hydroxyl groups, leading to the formation of AgNPs, and providing stability for the newly formed nanoparticles.

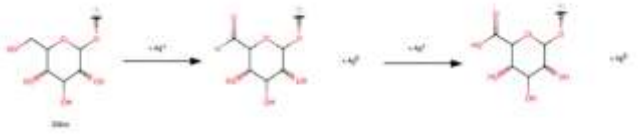

Figure 2: Phyto-reduction of Ag^+ to AgNPs by saponins (glycones).

Other secondary metabolites detected in SPE are flavonoids, tannins, and polyphenols. The hydroxy groups in the three classes of compounds work as reductants and form quinones, while Ag+ is reduced to Ag^0 (Thongnopkun et al., 2018). For example, the reaction mechanism in flavonoid compounds can be seen in Figure 3.

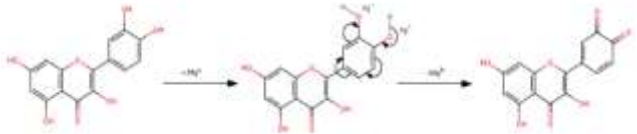

Figure 3. Phyto-reduction of Ag^+ to Ag^0 by flavonoid group compounds

The functional groups in AgNPs solution can be determined using FTIR method and the identification is carried out by analyzing the existing peaks. **Figure 4** shows that there are several peaks in FTIR spectrum of the AgNPs solution.

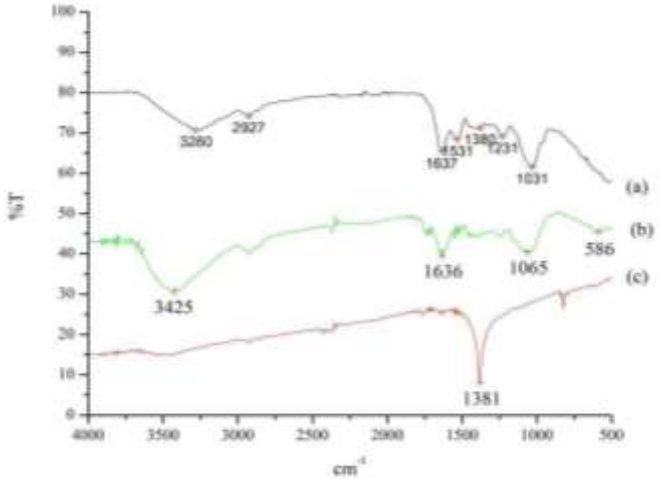

Figure 4. FTIR spectra of AgNPs solution (a), SPE (b), $AgNO_3$ solution (c).

The identification results of the spectrum peaks in Figure 4 can be seen in Table 2. The data shows an increase in the intensity of O-H functional group in AgNPs solution when compared to salak peel extract. This increase in intensity also represents the number of O-H groups derived from the oxidation process. In addition, the appearance of a second C=O bond in AgNPs solution (1531 cm^{-1}) shows the formation of quinones or conjugated ketones, serving as the end products of the oxidation process in flavonoids, tannins, and polyphenols.

Table 2. Functional groups found in AgNPs Solution, SPE, and $AgNO_3$ Solution

$AgNO_3$ Solution			SPE			AgNPs Solution		
Wave number (cm^{-1})	Intensisty	Functional Group	Wave number (cm^{-1})	Intensisty	Functional Group	Wave number (cm^{-1})	Intensisty	Functional Group
1381	0.12	C-H	3425	9.5	O-H	3280	11.08	O-H
			1636	23.22	C=O	2927	7.59	C-H
			1065	19.18	C-N	1637	16.52	C=O
			586	24.27	C-X	1531	13.58	C=O
						1380	10.58	C-H
						1231	12.65	C-O
						1031	20.22	C-X

The quinones formed have a role as stabilizers of AgNPs produced through free electron pairs and pi electrons from the quinone structure. This was stated by Suresh et al., (2021) when researching the synthesis of AgNPs using *Terminalia cuneata* shown in Figure 5.

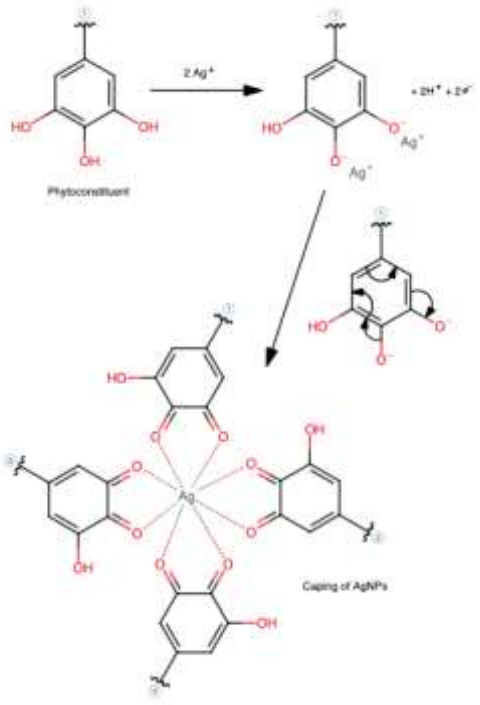

Figure 5. Schematic of phytochemicals (secondary metabolites) as capping agents for AgNPs formation.

During the synthesis process, the extracted biomolecules also serve as capping agents (Prasetyaningtyas et al., 2020). The capping process refers to the incorporation of biomolecules on the surface of AgNPs to avoid agglomeration. Biomolecules perform the capping process and the carbonyl groups of amino acids have a strong ability to bind metal ions. The nanoencapsulation surrounding these nanoparticles forms a membrane-like protective layer to avoid agglomeration.

The optimized conditions were temperature, $AgNO_3$ concentration and synthesis time (Figure 6). Several variations of $AgNO_3$ concentration used were 0.25 Mm, 1.00 Mm, 2.00 Mm, and 4.00 mM. Each mixing of salak peel extract with a concentration of $AgNO_3$ produces a change in the color of the solution whose absorbance and wavelength can be observed using a UV-Vis spectrophotometer. The expected AgNPs solution has typical absorption (surface plasmon resonance = SPR) at a wavelength of 400 nm-450 nm [49].

a b c d e

Figure 6: SPE (a), $AgNO_3$ solution 0.25 mM (b), 1.00 mM (c), 2.00 mM (d), 4.00 mM, and (e) 30 °C for 30 minutes.

The AgNPs solution was incubated for 5, 15, 30, 60, 120, 240, and 1440 minutes to determine the absorption intensity (Figure 7). The wavelength of the maximum absorbance ranged from 410 nm to 423 nm. The SPR observed from 5 to 240 minutes is relatively the same intensity, while at 1440 minutes there is a wavelength shift towards a higher intensity. At maximum absorbance, this shift occurs due to differences in the size and shape of the nanoparticles. A red and blue shift shows a larger and smaller particle size (Vanlalveni et al., 2021), while silver nitrate is a precursor to the formation of AgNPs. The optimal synthesis of AgNPs was found at a temperature of 30 °C with an incubation time of 30 minutes and an $AgNO_3$ concentration of 1 mM.

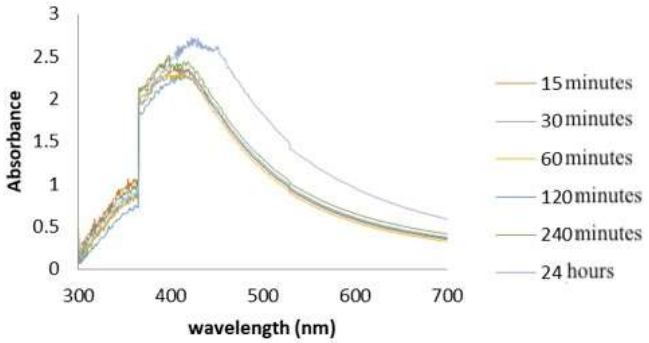

Figure 7. Absorbance measurement of AgNPs solution using UV-Vis spectrophotometer based on incubation time at a concentration of 1.00 mM and a temperature of 30 °C.

The synthesis temperature of AgNPs also affects the color of the mixture and the absorption peak of silver nanoparticles. The heating temperatures used were 30 °C, 50 °C, and 75 °C, as reported in Figure 8. At a temperature of 30 °C, the alteration in color of the mixture occurs gradually. Conversely, the reduction process increases when the mixture is heated to 50 °C and 75 °C, resulting in a quicker transition to a dark brown color.

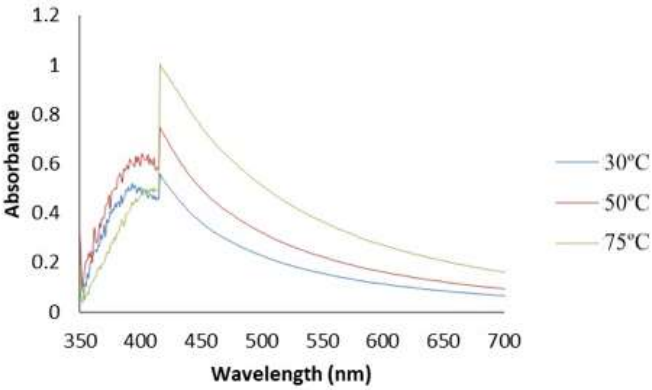

Figure 8. Absorbance measurement results of AgNPs solution using UV-Vis spectrophotometer based on incubation temperature at a concentration of 1.00 mM and incubation time of 30 minutes.

The wavelength showing the maximum absorbance is recorded at 423 nm and the distinguishing factor is in the intensity of the absorbance, with the highest observed at 75 °C. The difference in intensity is caused by the number of AgNPs particles formed.

Another factor affecting the color change of AgNPs synthesis is the concentration of $AgNO_3$. At a concentration of 0.25 Mm, the solution did not change color and no absorption was reported due to the slow formation of AgNPs particles. The concentrations of 1.00 Mm, 2.00 Mm, and 4.00 mM experienced a color change and reported absorption as particle formation of AgNPs, as reported in Figure 9.

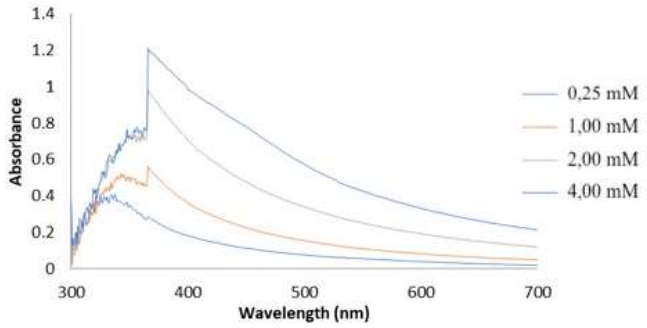

Figure 9. The absorbance measurement results of AgNPs solution based on different concentrations using UV-Vis spectrophotometer at 30 °C and 30 minutes incubation time.

The solution incubated for 30 minutes at 30 °C with an $AgNO_3$ concentration of 1.00 mM was measured using a UV-Vis Spectrophotometer in the wavelength range of 300 - 700 nm. The nanoparticles were observed to determine the surface plasmon resonance (SPR) of AgNPs differentiated at a wavelength of 410 nm with an absorbance of 2.361 (Prodjosantoso et al., 2019). The stability of AgNPs at the incubation time can be seen from UV-Vis absorption peak in the range of 350 nm - 450 nm under the characteristics of SPR.

Conclusion

In conclusion, snake fruit peel was reported to contain alkaloid, flavonoid, saponin, polyphenol, and tannins compounds. The optimal green synthesis of AgNPs using SPE was obtained at an $AgNO_3$ concentration of 1.00 mM with a temperature of 30 °C and an incubation time of 30 minutes.

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Author Contributions
Conceptualization, Dewi K. A. Kusumahastuti, Margareta N. Cahyanti, November R. Aminu.; methodology, Dewi K. A. Kusumahastuti, Margareta N. Cahyanti, and November R. Aminu.; validation, Dewi K. A. Kusumahastuti.; formal analysis, Dewi K. A. Kusumahastuti, Margareta N. Cahyanti, November R. Aminu.; resources, Dewi K. A. Kusumahastuti, November R. Aminu.; data curation, Margareta N. Cahyanti, Jumiyati; writing—original draft preparation, Dewi K. A. Kusumahastuti, Jumiyati.; writing—review and editing, Margareta N. Cahyanti, November R. Aminu.; visualization, November R. Aminu.; supervision, Dewi K. A. Kusumahastuti.; project administration, Margareta N. Cahyanti.; funding acquisition, Dewi K. A. Kusumahastuti. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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