Impact of temperature and pH on antioxidant activity of green silver nanoparticles fabricated from *Ananas comosus* peel extracts

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Abstract. Silver nanoparticles arose as a new weapon in the development of green synthesis of these nanoparticles using diverse natural resources such as plant leaf, microorganisms, and fruit extracts. Biosynthesis approach by using waste materials from plant is widely used as it is proven to be environmentally and cost friendly method. This study aims to identify the impact of temperature and pH on antioxidant activities of silver nanoparticles fabricated from pineapple (*Ananas comosus*) peel extract. Different temperature (70°C, 80°C and 90°C) and pH (7, 8 and 9) have been tested to the samples and characterized using UV-Visible Spectroscopy. Then, antioxidant activities of the AgNPs produced using different temperature and pH were determined using free radical scavenging ability on 2,2-diphenyl-2-picrylhydrazyl (DPPH). The results were found at pH 9, the surface plasmon resonance peak for biosynthesized AgNPs was at 425 nm on Day 2 while other treatments took longer time to exhibit the AgNPs peak. Biosynthesized AgNPs treated at temperature 90°C showed the optimum temperature when it exhibited peak at 420 nm on Day 3. The biosynthesized of AgNPs from pineapple peel extracts exhibited potential antioxidant activity in the DPPH scavenging by highest DPPH scavenging percentage is at 84.75% for AgNPs treated at pH 9 and 80.29 % for AgNPs treated at 90°C with concentration of 1000 µg/mL. Temperature and pH gave significant impact in synthesis and increase antioxidant activity of green silver nanoparticles.

Keywords: Temperature, pH, Antioxidant activity, Silver nanoparticles, *Ananas comosus*

1. Introduction
Nanotechnology is an emerging area with wide collection of biomedical usage involving the use of silver nanoparticles (AgNPs) as a substitute for antimicrobial agents [1]. The main successful studied nanoparticles are from the noble metals such as silver, gold and platinum. Nanotechnology is the study and use of structures ranging from size of 0.1 nm to 100 [2]. The nanoparticles have potential applications in all fields of sciences and technology due to excellent physicochemical properties [3].
Silver nanoparticles formed when Ag⁺ ions dissociate from a silver compound when it is dissolved and gain an electron in an oxidation-reduction reaction with a reducing agent. Silver nanoparticles have many applications due to the large degree of commercialization such as products for wound treatment [4]. Silver in the product could act as an antimicrobial agent [5]. In modern times, lots of methodologies were used to prepare the nanoscale silver particles. For examples are electrochemical, sonochemical and microwave-assisted process. However, these approaches are hard for purification because of hazardous and high energy chemicals.

Green synthesis is defined as the usage of environmentally compatible materials such as bacteria, fungi and plants in the synthesis of nanoparticles [6]. This green synthesis is free of short falls from the conventional methods [7]. As a substitute to the conventional method, biosynthesized from extracts suggest many benefits. Biological methods are less pricey, less toxicity and sustainable for biosynthesis of silver nanoparticles from various microorganisms and plants [8]. Large scale production, easiness, economically feasible nature, and eco-friendliness equaled to the other prevailing approaches such as using fungi and bacteria which would not be possible with the controversial conventional ways for the production of nanoparticles [9].

Temperature plays vital part to affect the nucleation process of nanoparticle formation. pH plays a significant role in synthesizing silver nanoparticles [5]. pH induces the reactivity of the extracts with silver ions. Thus, the change in pH brought significant change in yield. *Ananas comosus* has many medicinal values. *A. comosus* or well known as pineapple acts as extra nutritious fruit for decent health enrich with vitamin C, manganese, and fibre [10]. From the research, revealed the crude extracts and purified fractions of *A. comosus* have the antioxidant properties as the crude extract of stem had powerful antioxidant activity (70.2%) compared with the other extracts [11]. These compounds hinder and prevent the molecules oxidation by hindering the beginning or spreading of oxidative chain reaction.

Therefore, in this study aim to synthesize silver nanoparticle fabricated with peel extract of *Ananas comosus*, characterized using UV-visible spectroscopy and determine their antioxidant activity effect from temperature and pH. Comparison effect of both factors in an antioxidant activity was analysed.

2. Methodology

2.1. Material

The raw materials were used in the experimental work were peel of *Ananas comosus*. The *Ananas comosus* was purchased from local fruit market in Arau, Perlis.

2.2 *Ananas comosus* extraction

The pineapple obtained was cleaned thoroughly with distilled water to discard any dirt. The peel of the pineapple was separated from its flesh and weighed 50 grams of both of pineapple waste. The extraction method was adopted with slight modifications [12]. The pineapple peel was dried in the oven at 50°C for 3 days. Later, the dried peel was ground into powder using an electric blender. About 30 g of the peel powder were weighed and moved into 1000 ml beakers that contains 600 ml of distilled water. The extract obtained was filtered using Whatman No 1 filter paper. Then, the filtrate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was gathered into a 500 ml Erlenmeyer flask to be cooled down to room temperature and stored for further use.

2.3 Synthesis of green silver nanoparticles

About 0.17 g of AgNO₃ was mixed into 1000 ml of distilled water inside a beaker to prepare an aqueous solution of (1mM) AgNO₃. The filtrate (5.0 mL) of *Ananas comosus* peel extract was added into 20 mL of 1mM aqueous AgNO₃.
2.4 Characterization of green silver nanoparticles

UV-Visible Spectrophotometer was used to carry out UV Visible measurements of the prepared nanoparticles of the pineapple peel extracts. The bio-reduction of Ag$^+$ ions to form AgNPs was examined. The sample absorption was scanned between 300 and 800 nm at a scanning speed of 300nm/min with a resolution of 1 nm. After diluting a small aliquot of the sample into deionized water, the reduction of pure Ag$^+$ ions were monitored by measuring the UV-Vis spectrum of reaction medium. 1 ml of the sample was pipetted into a test tube and diluted with 4 ml of deionized water and subsequently analysed at room temperature. The colour of the reaction solution was recorded at each time interval.

2.5 Determination of antioxidant properties on biosynthesized silver nanoparticles

2.5.1 Free Radical Scavenging Ability on 2,2-diphenyl-2-picrylhydrazyl (DPPH)

Different concentrations of extracts with silver nanoparticles, extracts and standard ascorbic acids ranging from 10µg/mL, 100µg/mL and 1000µg/mL were taken in different test tubes. The DPPH solution was prepared by dissolving 2 mg of DPPH in 100 mL of methanol. Later, 40 µL of samples with different concentrations were added into 4 mL of methanolic DPPH solution. Then, the solutions were shaken and incubated for 30 min in dark place. The absorbance of stable DPPH was recorded at 517 nm. The DPPH with no sample was used as a control. It was prepared using the same method. Free radical scavenging was expressed as the percentage of inhibition and the equation (3.2) was used to determine the percentage of scavenging activity.

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\%\text{DPPH Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}
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2.5.2 Statistical Analysis

The results of antioxidant activity assays were expressed as mean value and standard deviation on the triplicates samples. The data were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test SPSS software. (P<0.05).

3. Result and Discussion

3.1. Characterization of green synthesized silver nanoparticles (AgNPs) using UV-Visible Spectrophotometer

UV-Visible spectroscopic analysis of the silver nanoparticles (AgNPs) was performed by using Cary 50 UV-Vis Spectrophotometer to investigate the effect of temperature and pH. The prepared aqueous solution treated at different temperature and pH of AgNPs green synthesized from A. comosus peel extract showed different absorption peak band at various days. The Table 1 and 2 show wavelength reading from UV-Vis Spectra of AgNPs treated at different temperature and PH.
Table 1. The wavelength reading from UV-Vis Spectra of AgNPs Treated at Different Temperature

| AgNPs at Different Temperature (°C) | Day 1 Wavelength | Day 2 Wavelength | Day 3 Wavelength | Day 4 Wavelength |
|-------------------------------------|------------------|------------------|------------------|------------------|
| 70                                  | 215              | 250              | 380              | 422              |
| 80                                  | 220              | 266              | 386              | 425              |
| 90                                  | 300              | 415              | 420              | 428              |

The sample that was treated with temperature of 70 °C exhibited peak at only 215 nm on day 1 while the absorption peak increases to 422 nm as the incubation period increases up to day 4 as shown in Table 1. The sample that was treated with temperature of 80 °C exhibited peak at 220 nm on Day 1 and increases to 425 nm on Day 4. From the result, the sample treated with temperature of 90 °C exhibited peak of 300 nm on Day 1 and increase gradually up to 428 nm on Day 4. The weak absorption peak exhibited earlier indicated the presence of several organic compounds interact with silver ions into solution and imply a possible mechanism for the reduction of the silver nanoparticles. Therefore, it is clearly indicated that the sample treated at 90 °C has the optimum temperature of biosynthesized AgNPs as it exhibited the most absorption wavelength at Day 1 until Day 4 compared to other samples that took longer time to reach the desired AgNPs of wavelength. The higher temperature leads to an increase in the activation energy of the molecules, faster rate of reaction and decrease the size of nanoparticles [5]. Hence, temperature of 90 °C gave better AgNPs.

Table 2. The wavelength reading from UV-Vis Spectra of AgNPs Treated at Different pH

| AgNPs at Different pH | Day 1 Wavelength | Day 2 Wavelength | Day 3 Wavelength | Day 4 Wavelength |
|-----------------------|------------------|------------------|------------------|------------------|
| pH 7                  | 220              | 348              | 422              | 429              |
| pH 8                  | 265              | 370              | 430              | 430              |
| pH 9                  | 320              | 425              | 441              | 441              |

From Table 2, the sample that was treated with pH 7 exhibited peak at only 220 nm on Day 1 and increases to 429 nm on Day 4. For the sample that was treated at pH 8, on Day 1, the absorption peak displays 265 nm and increases up to 430 nm on Day 3 and Day 4. Lastly, the sample with pH 9 exhibited wavelength of 320 nm on Day 1 and increases to 441 nm on the Day 4. This shows that the higher the pH, the faster the rate of reaction of the AgNPs. From Table 4.5, it is clearly indicated that the optimum pH for AgNPs is at pH 9 as the sample has reach the peak of AgNPs wavelength which is at 425 nm and its remain stable to 441nm. The UV absorption peak of AgNPs occur on the range of 400 nm – 450 nm [13].

At higher pH, the large number of phenolic functional groups available for silver binding facilitated a higher number of Ag⁺ ions to bind [14]. From the study, the maximum wavelength was found to be for pH 9. This may be due to the presence of ferulic acid in Ananas comosus peel. The study on the pineapple peel waste confirmed the presence of ferulic acid in the pineapple [15]. In alkaline condition, ferulic acid reduces the silver nitrate into silver nanoparticles may be the reason for higher absorbance of silver nanoparticles at alkaline pH.

Most samples exhibited peak at only about 220 to 320 nm as shown in Table 1 and Table 2 on Day 1. It does not reach the typical band of AgNPs which is at 350 to 450 nm. However, as the incubation period increases up to Day 6, the samples gradually exhibited the desired band of AgNPs. The most optimum sample is at pH 9 where it exhibited the band of 441 nm on Day 3. The study
on *Ananas comosus* peel extracts display a characteristic absorption band of AgNPs at about 445 nm [12]. The bioactive compounds in plant have the ability to bind to the nanoparticles enhancing the stability of absorption [5].

*Ananas comosus* contains numerous phytochemicals that have biological properties such as antioxidative, antimicrobial, and anti-inflammatory applications [15]. Thus, the peel extracts of pineapple might have the same mentioned bioactive compounds. These bioactive compounds could be dependable for the bioreduction and many active potential effects of the biosynthesized silver nanoparticles. The plant-mediated biosynthesis of AgNPs is a straightforward practice, highly effective and with short reaction times due to the presence of numerous metabolites (phenols, ketones, proteins, aldehydes, amides, carboxylic acids), therefore plants have the ability to reduce and stabilize nanoparticles [1]. A capping and effective stabilization was due to the presence of possible biomolecules present in the peel extracts [16]. They claimed that the capping material reduction would lead to the reduction of silver nanoparticles as the bioactive compounds found in the pineapple’s wastes would act as capping agent.

Figure 1 and Table 3 showed the antioxidant activity of different concentration ranging of aqueous *A. comosus* peel extracts, different AgNPs from peel extracts at 70°C, 80°C and 90°C and the ascorbic acid that act as standard.

**Table 3.** Antioxidant activity of different concentration of AgNPs treated at different temperature

| Samples         | 0.1 µg/mL | 1 µg/mL | 10 µg/mL | 100 µg/mL | 1000 µg/mL |
|-----------------|-----------|---------|----------|-----------|------------|
| Peel extracts   | 31.86±0.21| 45.31±0.08| 67.37±0.11|           |            |
| AgNPs (70°C)    | 40.50±0.20| 50.33±0.09| 72.33±0.11|           |            |
| AgNPs (80°C)    | 43.46±0.14| 56.76±0.05| 76.26±0.06|           |            |
| AgNPs (90°C)    | 46.93±0.05| 58.53±0.73| 80.30±0.10|           |            |
| Ascorbic Acid   | 23.58±0.09| 41.91±0.05| 60.31±0.07| 78.79±0.16| 93.56±0.08 |

**Figure 1.** The Antioxidant Activity of Aqueous *A. comosus* Peel Extracts, Different AgNPs at 70°C, 80°C and 90°C and The Ascorbic Acid
From Figure 1, the silver nanoparticles at different concentration exhibit higher scavenging activity than to the peel extracts. The higher the concentrations of the samples, the higher the scavenging percentage would be. The synthesized silver nanoparticles at 90°C have the highest percentage of DPPH which is 80.3% with concentration of 1000µg/mL. For concentration of 100µg/mL, the silver nanoparticle has the ability to scavenge the DPPH for 58.53% and 46.93% at 100µg/mL. While for the synthesized silver nanoparticles at 80°C, the percentage of DPPH is at 76.26% with concentration of 1000µg/mL. For concentration of 100µg/mL, the silver nanoparticle has the ability to scavenge the DPPH for 56.76% and 43.46% at 100µg/mL. For the synthesized silver nanoparticles at 70°C, the percentage of DPPH scavenging is at 72.33% with concentration of 1000µg/mL. For concentration of 100µg/mL, the silver nanoparticle has the ability to scavenge the DPPH for 50.33% and 40.50% at 100µg/mL.

Figure 2 and Table 4 showed the antioxidant activity of different concentration ranging of aqueous A. comosus peel extracts, different AgNPs from peel extracts at pH 7, pH 8 and pH 9 and the ascorbic acid that act as standard.

**Table 4. Antioxidant activity of different concentration of AgNPs treated at pH**

| Samples            | 0.1 µg/mL | 1 µg/mL | 10 µg/mL | 100 µg/mL | 1000 µg/mL |
|--------------------|-----------|---------|----------|-----------|------------|
| Peel extracts      | 31.86±0.21| 45.31±0.08| 67.37±0.11|           |
| AgNPs (pH 7)       | 42.87±0.07| 54.18±3.55| 75.79±0.11|           |
| AgNPs (pH 8)       | 47.97±0.04| 60.98±0.06| 78.36±0.08|           |
| AgNPs (pH 9)       | 51.59±0.03| 66.15±1.65| 84.75±0.10|           |
| Ascorbic Acid      | 23.58±0.09| 41.91±0.05| 60.31±0.07| 78.79±0.16| 93.56±0.08 |

Based on the Figure 2, the silver nanoparticles at different concentration exhibit higher scavenging activity compared to the peel extracts. The higher the concentrations of the samples, the higher the scavenging percentage would be. The synthesized silver nanoparticles at pH 9 have the highest percentage of DPPH which is 84.75% with concentration of 1000µg/mL. For concentration of 100µg/mL, the silver nanoparticle has the ability to scavenge the DPPH for 66.15% and 51.59% at 100µg/mL. Moreover, for the synthesized silver nanoparticles at pH 8, the percentage of DPPH...
scavenging is at 78.36% with concentration of 1000µg/mL. For concentration of 100µg/mL, the silver nanoparticle has the ability to scavenge the DPPH for 60.98% and 47.97% at 100µg/mL. On top of that, for the synthesized silver nanoparticles at pH 7, the percentage of DPPH scavenging is at 75.79% with concentration of 1000µg/mL. For concentration of 100µg/mL, the silver nanoparticle has the ability to scavenge the DPPH for 54.18% and 42.87% at 100µg/mL.

The silver nanoparticles exhibited higher scavenging activity for DPPH assay due to the integration of additional oxidants onto the surface of silver nanoparticles owing to a large surface zone [15]. Moreover, the properties of molecule that coated the surface of the metallic nanoparticles influenced the antioxidant properties [17]. Thus, it is vital to determine either bioactive molecule found in *Ananas comosus* peel extracts still remain maintained by the biosynthesized AgNPs.

The optimum scavenging activities of all the samples were determined at the concentrations of 1000 µg/mL because pineapple peel extract contain abundance of phenolic compound. The antioxidant activity was due to capped phenolic compounds found in the extracts [18]. Phenolic group helps to convert silver nitrate to AgNPs because of its electron donating ability. Moreover, the improved property of AgNPs is also due to the simultaneous activity of polyphenols as antioxidant agents and AgNPs as a catalyst [19].

In addition, this present study showed that higher rapid in synthesis of nanoparticles at various pH and temperature would result in higher percentage of DPPH radical scavenging. An increasing in temperature, the DPPH scavenging activity becomes stable [20]. DPPH radical scavenging activity increased when pH and temperature increases because different peptides would have different proper pH that would lead to high bioactivity. They stated that at higher pH, it will promote the amino-group ionization from amino acids and peptides. This will increase the H⁺ release and enhance more the radical scavenging.

4. Conclusion

As a conclusion, high temperature and moderately alkaline of pH play an impactful role in synthesis and physio-activity of green silver nanoparticles. The treatment at pH 9 and temperature of 90°C showed the optimum treatments for surface plasmon resonance peak of biosynthesized AgNPs and exhibited potential free radical scavenging.

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