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# Phytogenic silver nanoparticle (AgNP) from *Ananas comosus (L) Merr.* peel extract to inhibiting the pathogen resistance

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# Phytogenic silver nanoparticle (AgNP) from *Ananas comosus (L) Merr.* peel extract to inhibiting the pathogen resistance

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**Abstract**. Green Nanotechnology utilizing various plant extracts (phytogenic) has widely known and proven as ecofriendly and inexpensive scientific approach with numerous potentials in medical treatments. Silver nanoparticles (AgNPs) has been commonly used in home cleaning products, wound dressing and antimicrobial coating for medical equipment. Furthermore, Ananas comosus (L.) Merr. (AC) is an exotic tropical fruit, found abundantly in Riau Province. It is rich on bromelain enzyme in addition to its potential as antibacterial, antioxidant as well as anticancer. Contrarily, utilization of waste from plants, fruits and food as alternative natural reduction and capping agents from metal ion nanoparticles is still being developed. Combining these two elements has an immense potential as an effective alternative therapeutic substance in the near future. The purpose of this research is to analyze AgNPs from AC by characterizing the product of biosynthesis. Furthermore, this research will also observe antimicrobial activity of AC-AgNPs against Methicillin Resistant Staphylococcus aureus (MRSA). As a result, the biosynthesis produced AC-AgNPs which showed the ability to inhibit the growth of MRSA with an average of 10.59 mm, 20% more effective compared to pure AC extract. AC-AgNPs formation were characterized using UV–visible spectroscopy in the interval of 439– 486 nm.

## 1. Introduction
Nanoparticles are the simplest structural design with a size range of 1–100 nm. Nanoparticles have various uniqueness, such as a greater ratio of surface area to volume and higher surface energy [1-4] Nanoparticles also display completely new properties due to the specific characteristics of their particle size, shape and distribution. Nanoparticle synthesis can be formed through two methods, namely top-down or bottom-up[5]. In the top-down process, nanoparticles are generated through degradation of the size of the original material. In contrast, in a bottom-up process, nanoparticles are produced from the smallest size at the atomic level [6]. Nanoparticle biosynthesis itself is included in the category of bottom-up processes, where bioreductant against metal ions is carried out by extra and intracellular secondary metabolites from plants and microbes.

Silver nanoparticles (AgNP) themselves are known for their various antidiabetic, antioxidant, antibacterial, and cytotoxic activities [7-9]. Since the last few years, silver nanoparticles have been utilized in various industries for food, agriculture, medical needs, drug administration, etc. [10-11]. Evaluation of AgNP results from the Aeromonas sp supernatant culture. THG-FG1.2 has been shown to prevent the development of bacterial strains that are fully insensitive to different antibiotics, such as erythromycin, lincomycin, novobiocin, penicillin G, vancomycin, and oleandomycin [12-13].

Meanwhile, Ananas comosus (L.) Merr. (AC) or Pineapple is the Bromeliaceae family which is the most popular for consumption. Traditionally, pineapple has been used as medicine [14-15]. Pineapple fruit is usually consumed directly, and the skin is only a byproduct (waste). The use of pineapple peel as a therapeutic material has the potential to improve the commercial value of pineapple fruit. Other than that, pineapple peel contains rich nutrients and bioactive compounds such as vitamins, minerals, lipids, vitamin C, phenolic compounds, flavonoids, carotenoids, etc [8-15]. These bioactive compounds can be an alternative to metal ion reductants, as well as stabilize metal nanoparticles from natural agglomeration processes [8-16].

The aim of this study was to investigate the biosynthesis of silver nanoparticles (AgNP) from the peel extract of Ananas comosus (L) Merr (AC), a waste from the abundant production of pineapple in the province of Riau. Characterization of silver nanoparticles (AC-AgNP) made. Then, look at the antimicrobial activity of AC-AgNP against bacterial pathogenic resistance.

## 2. Materials and methods

### *2.1 Materials and preparation of the Ananas comosus (AC) peel extract*

The peel of *Ananas comosus (L.) Merr* pineapple was obtained from a local fruit seller in Pekanbaru City, Riau Province. Meanwhile, the chemicals used in this study, such as Silver Nitrate ($AgNO_3$), were purchased from Sigma-Aldrich. Then, the pineapple skin is cut into small pieces and dried using tissue paper. Pineapple rind pieces (total weight 120g) soaked in 550 mL DO (Deionized water) and boiled for 20-30 minutes under constant stirring conditions. After boiling, the solution was cooled and filtered using Whatman No.1 filter paper. Furthermore, the filtrate was stored at 4˚C until further use.

### *2.2 Synthesis of AgNPs using extracts of AC-AgNPs*

AgNP biosynthesis was carried out triplo. A total of 10 mL of pineapple peel extract (AC) was dropped into 3 sets of 100 mL 1mM $AgNO_3$ solution in a 250 mL Erlenmeyer flask. The solution was then stirred with magnetic stirrer at room temperature for 24 hours [26, 27]. The biosynthetic AgNP solution was then centrifuged at 10,000 rpm for 30 minutes. The centrifuged supernatant was discarded, while the pellets were washed 3-4 times with distilled water, and then centrifuged once again to remove any remaining unbound AC extract. Furthermore, the centrifuged pellets were dried at 55 C in an airtight bottle for the next research stage.

### *2.3 UV–visible spectroscopy characterization of AC-AgNPs*

Bioreduction of $Ag^+$ to $Ag^0$ ions was analyzed by measuring UV-Vis spectroscopy for 24 hours of stirring, with a resolution of 2 nm over a wavelength range of 300-700 nm. UV-Vis spectroscopy analysis was performed at 0, 1, 3, 6, 12, 18 and 24 hour intervals during the stirring period to track the formation of silver nanoparticles that appeared at the peak of absorption. 350–650 nm. The color of the reaction solution is also recorded at each time interval [17]

### *2.4 Antibacterial action of biosynthesized AC-AgNPs*

Silver nanoparticle activity test was carried out according to AATCC 100-1999 standard. The type of bacteria used is Methicillin-Resistant Staphylococcus aureus (MRSA). Qualitative antimicrobial activity testing or microbial inhibition test is carried out by making a series of dilution test compounds (with dilution variations of 25%, 50% and 100%). Gentamicin standard antibiotics (50 µg / disc) were taken as positive control and 5% NaCl were taken as negative control. The disc was then applied to the MRSA culture by the Kirby-Bauer method[18].

## 3. Results and Discussion

### *3.1 Synthesis of AgNPs using Ananas comosus (L.) Merr peel extract*

In this experiment, AC-AgNPs biosynthesis was conducted in a controlled environment using pineapple peel extracted from Ananas comosus (L.) Merr (Figure. 1 and Figure. 2), which was collected from the distribution of pineapple in Pekanbaru City Market. Biosynthesis of silver nanoparticles reveals a significant shift of color from transparent to reddish brown

**Figure 1.** Biosynthesis silver nano particles (a) *Ananas comosus* extract

**Figure 2.** A gradual change in the color of AC-AgNPs

### *3.2 UV–visible spectroscopy characterization of AC-AgNPs*

Based on the visualization of the detected color change through spectral analysis of UV-Vis spectrometer, the results of the change in the color gradation of the solution during the 24-hour stirring interval were obtained. Where the highest AC-AgNPs absorbance peak was detected at a wavelength of 439 nm to 486 nm. As a Figure. 3 below, silver nanoparticles is formed at an absorbance peak interval of 350–650 nm (Kumar et al., 2019). Referring to the method of estimating the concentration of capped citrate silver nanoparticles from the UV-Vis spectrum, the diameter size of the silver AC-AgNPs nanoparticles obtained ranges from 60 - 100 d / nm [19].

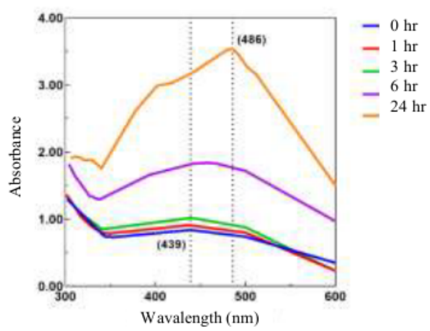


**Figure 3.** UV-vis result of Silver Nanoparticles *Ananas comosus (L.) Merr* peel extract (AC-AgNP)

### *3.3 Antibacterial resistance activity of phytogenic of AC-AgNPs extracts*

Qualitative antimicrobial activity testing or microbial inhibition test was carried out by making a series of comparisons between several treatments of stirring time (Figure. 4). The standard antibiotic vancomycin (20 µg / disc) was taken as a positive control and distilled water as a negative control. The disc was then applied to MRSA cultures using the Kirby-bauer method [20].

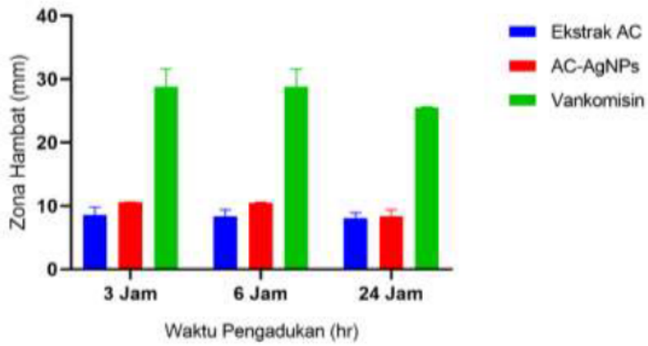


**Figure 4.** Antibacterial resistance potential of the biosynthesized AC-AgNPs to inhibit MRSA

After the Kruskal Wallis test was carried out in the stirring time group, the P value $< 0.05$ was obtained, which means that there is a difference in the diameter of the stirring zone between stirring times. Where the greatest zone of inhibition was found in the stirring time group for 3 hours and 6 hours, with an average diameter of 10.593 mm in the zone. Meanwhile, the mean difference of inhibition zone to pure AC (A.comosus) extract without silver nanoparticles was 2.00 mm greater and 3.50 mm lower than the positive control group (vancomycin).

### *3.4 Discussion*

This visible shift in color were followed by the increase in absorbency by liquid AC-AgNPs. Where the highest AC-AgNPs absorbance peak was detected at a wavelength of 439 nm to 486 nm. Where it is known that a solution of silver nanoparticles is formed at the absorbance peak interval 350–650 nm. Referring to the method of estimating the concentration of silver nanoparticles capped citrate from the UV-Vis spectrum, the diameter size of the silver AC-AgNPs nanoparticles obtained ranges from 60 - 100 d / nm. [19-21]. Assessment of antimicrobial activity showed the effectivity of silver nanoparticle solution inhibition AC-AgNPs towards MRSA bacteria were found in the stirring time group for 3 hours and 6 hours, with an average diameter of 10.593 mm in the zone. Meanwhile, the mean difference of inhibition zone to pure AC (A.comosus) extract without silver nanoparticles was 2.00 mm greater and 3.50 mm lower than the positive control group (vancomycin).

## 4 Conclusions

AC-AgNPs have been shown to be effectively biosynthesized using a green nanotechnology process. The synthesis method was eco-friendly and cost-effective, since it includes plant extracts which are readily available. The higher content of flavonoids and phenolic acids found in both AC peel extracts may have facilitated the rapid reduction of $Ag^+$ ions and improved stability of AgNPs nanoparticles. AC-AgNP also demonstrated mild antibacterial activity. The findings of the current study indicated that AC-AgNP could prove to be an alternative antibiotic ingredient for various biomedical applications in the near future.

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