Green synthesis of silver nanoparticle using rambutan (*Nephelium lappaceum L.*) peel extract and its antibacterial activity against *Salmonella parathypi A.*

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Abstract. Ag nanoparticles (AgNPs) have been synthesized via green method using rambutan (*Nephelium lappaceum L.*) peel (RP) extract. RP extract was prepared by washing the RP using tap water thoroughly and boiling it in distilled water at 70°C for 60 min. RP extract and $AgNO_3$ were used as the starting materials for the synthesis of AgNPs. RP extract was added to 10^{-3} M $AgNO_3$ solution with a ratio by volume of 1:10 (RP : $AgNO_3$), stirred at room temperature. The solution's color changes from reddish to dark brown, indicating the reduction of Ag^+ in the solution. The synthesized AgNPs were characterized using UV-Visible Spectrophotometer, FTIR Spectrophotometer, and Scanning Electron Microscopy-Energy-dispersive X-ray Spectroscopy (SEM-EDS) instruments. UV-Visible spectra show that the AgNPs have the maximum absorption band at 450 nm which is typical for AgNPs. The FTIR spectra revealed that the protein in RP extract acts as the capping agent for the synthesized AgNPs. The synthesized AgNPs were tested for their antibacterial activity against *Salmonella parathypi A.* The antibacterial test shows that 50 µL of AgNPs resulted in the inhibition zone of 4 mm against the aforementioned microorganism.

1 Introduction

Nanoparticles are known as particles with the size ranging from 1 to 100 nm [1]. Recently, nanoparticles have obtained great interest due to their unique properties. The physical and chemical properties of metallic nanoparticles are different with bulk metals. Metallic nanoparticles have lower melting points, higher specific surface areas, specific optical properties, mechanical strengths and specific magnetizations [2]. Nanoparticles have been widely applied in many areas such as medicine, chemistry, pharmacy, industry, environment, biology, etc. One of the uses of nanoparticles is as antibacterial agents. Among all metal nanoparticles, silver nanoparticles (AgNPs) show the highest antibacterial activity. Despite the fact that the toxicity of AgNPs is still not clear, AgNPs have been applied as anti bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications [3].

The synthesis of AgNPs can be done using physical or chemical approaches. The physical approach involves some methods such as evaporation/condensation and laser ablation. The chemical approach uses strong reductants such as boro hydrate, citrate, ascorbate and elemental hydrogen. The reductants will reduce silver ions (Ag^+) to elemental form (Ag^0) which will eventually form the colloidal Ag particles [4]. Among these two approaches, chemical approach is more preferable. However, the chemical approach uses chemicals which are toxic and hazardous to the environment. Therefore, the development of the green process for the synthesis of AgNPs has been an emerging field of research interest. The green synthesis of AgNPs uses many types of plant extracts, bacteria, and fungi to eliminate the toxic chemicals needed for reducing the silver ions. The use of plant extract is most popular due to its simplicity and low cost [5]. The synthesis of AgNPs using plant extract is a rapid, eco-friendly, non-toxic and providing only a single step technique for the synthesis process [6].

Some plants' extracts have been reported to be successfully used for the synthesis of AgNPs. Other plants' extract which have been reported to be used for the synthesis of AgNPs are tea extract [7], inflorescence of *Cocous nucifera* [8], peel of *Citrus sinensis* [9], peel of *Musa paradisiacal* [10], leaves of *Carica papaya* [11], fruit of *Vitis vinifera* [12] and many other.

In this work, a native Indonesian plant, rambutan (*Nepphelium lappaceum*) peel extract was used to synthesize AgNPs. The synthesized AgNPs will then be studied for their anti bacterial activity against *Salmonella parathypi A*.

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2 Experimental Methods

2.1 Chemicals

Rambutan peel (RP) extract and $AgNO_3$ were used as starting materials for the synthesis of Ag nanoparticles (AgNPs) in this work. RP was washed using tap water and cut into pieces. RP was boiled in distilled water at 70°C for 1 h to obtain the RP extract. The RP extract was then filtered using Whatmann No.42. The extract was stored at 4°C for further use. The storage time for RP extract was 10 days at maximum. The chemicals used in this work were E.Merck production and were directly used without further purification. *Salmonella parathypi A*. strain was supplied by *Balai Laboratorium Kesehatan*, Yogyakarta. The distilled water was used as the solvent in this work.

2.2 Green Synthesis of Ag Nanoparticles (AgNPs)

The aqueous $AgNO_3$ solution with a concentration of 10^{-3} M was prepared in an Erlenmeyer flask. The RP extract with the ratio by volume of 1:10 (RP : AgNO3) was added to the solution to reduce the Ag^+ ions. The mixture was stirred at room temperature and the color change was observed. The AgNPs were considered to be formed when the color of the mixture changes into dark brown. The synthesized AgNPs were then characterized using UV-Visible Spectrophotometry, FTIR spectrophotometry, and Scanning Electron Microscopy-Energy-dispersive X-ray Spectroscopy (SEM-EDS).

2.3 Antibacterial Activity of AgNPs Against Salmonella parathypi A.

The synthesized AgNPs were tested for their antibacterial activity against *Salmonella parathypi A*. The antibacterial activity of AgNPs was studied using agar well diffusion method. The antibacterial activity of RP extract and chloramphenicol were used as comparisons. Agar media was prepared in a petri disk and added by the microorganism culture. The agar was divided into 4 parts and a well with a known diameter was prepared on each part of the agar. Well A was left blank, well B was filled with 50 µL RP extract, well C was filled with 50 µL AgNPs and well D was filled with 50 µL of 40 ppm Chloramphenicol and let to diffuse for some time. The petri disk was covered by paper and then incubated for 20 h. The inhibition zone for each substance was observed and measured.

3 Results and Discussion

3.1 Characterization of AgNPs

3.1.1 UV-Visible Spectrophotometry

In the synthesis of AgNPs using RP extract, the color of the extract changed from reddish to dark brown due to the reduction of Ag^+ in the solution. Fig 1 shows the UV-Visible spectra of RP extract, $AgNO_3$ solution, and the synthesized AgNPs. The maximum absorption band of AgNPs in this work is 450 nm. The region of 350-450 nm is typical for the maximum absorption band of AgNPs [13]. The broad and asymmetrical peak of the UV-Visible absorption band of the synthesized AgNPs indicates AgNPs with a broad interval of size and non-uniform shape. This work focuses on the potential of RP extract for the green synthesis of AgNPs and its antibacterial activity against Salmonella parathypi A. Therefore, the size and shape of AgNPs were not controlled in this study.

3.1.2 Scanning Electron Microscopy – Energy-dispersive X-Ray Spectroscopy (SEM – EDS)

The formation of AgNPs confirmed by UV-Visible spectra was further confirmed by analysis using SEM-EDS instrument. The result of SEM analysis and EDS spectra are presented in Fig 2. The SEM and EDS analyses confirm that the RP extract has been successfully used for synthesizing AgNPs. It is seen that the synthesized AgNPs have non-uniform shape and a wide range of particle size.

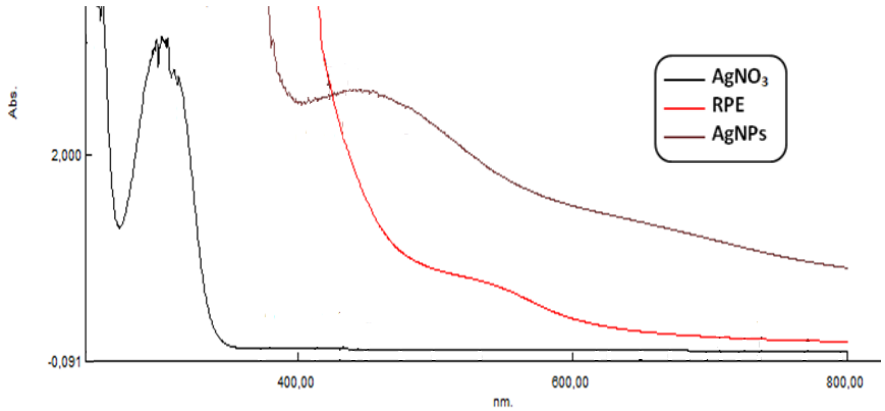
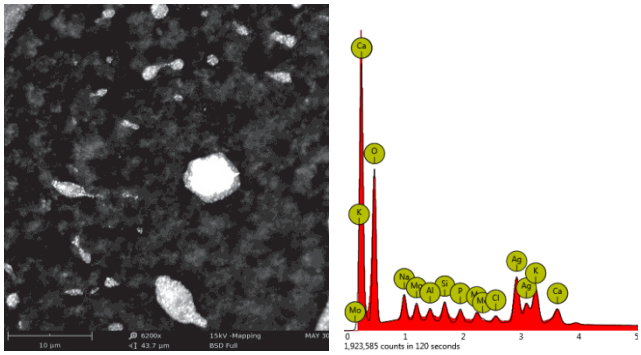


Fig. 1. UV Visible spectra of AgNPs synthesized using RP extract



(a) (b)

Fig. 2. (a) SEM analysis result of AgNPs; (b) EDS spectra of AgNPs

3.1.3 FTIR Spectrophotometry

FTIR analysis was conducted in order to know the functional groups of biomolecules in RP extract which are responsible for the synthesis and stabilization of AgNPs. The FTIR spectra of AgNPs synthesized using RP extract is displayed in Fig 3. The FTIR spectra of AgNPs shows some major peaks at 3339, 1574, 1322, 1039 and 820 cm^{-1}. The peak at 3339 cm^{-1} is attributed to O-H stretching of phenolic and alcoholic –OH groups. The absorption band at 1574 cm^{-1} corresponds to –COOH stretching vibration of flavonoids/phenolic groups. The peak at 1322 cm^{-1} is due to the stretching of C-O bond in aromatic groups. the absorption band at 1039 cm^{-1} is probably due to the C-N stretching of aromatic and aliphatic amines. It is known that the main biological active compounds of RP are anthocyanins, ellagitannins, ellagic acid, corilagin, geraniin, syringic acid and p-coumaric acid [14]. The presence of protein functional groups on the surface of AgNPs indicates that the proteins act as the capping agent for the synthesized AgNPs.

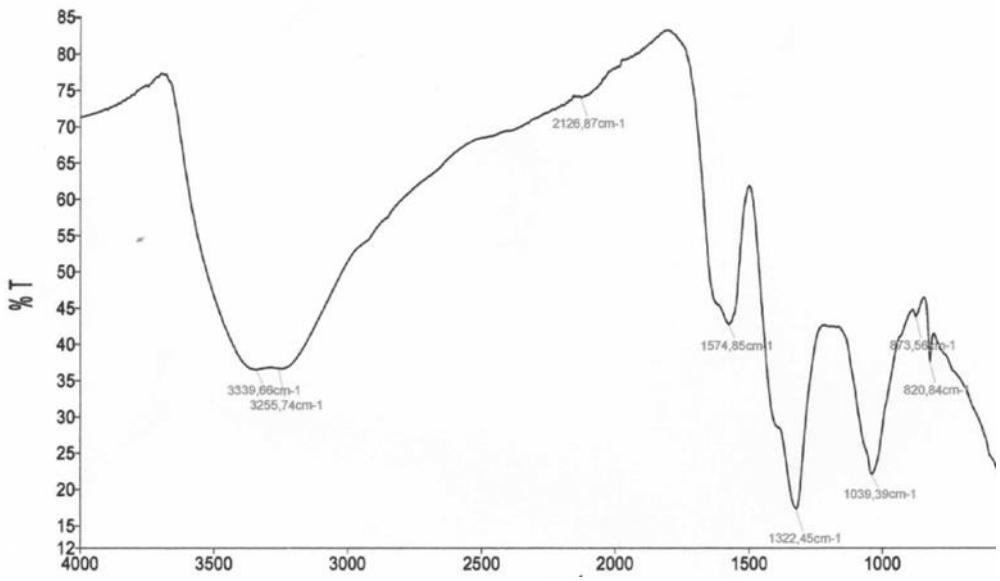


Fig. 3. FTIR spectra of the AgNPs synthesized using RP extrac

3.2 Antibacterial Activity of AgNPs Against *Salmonella parathypi A*.

Fig. 4 shows the result of the antibacterial test for each antibacterial agent. The inhibition zone of AgNPs, RP extract, and chloramphenicol against *Salmonella parathypi A*. is presented in Table 1. Among the three tested anti bacterial agent, Chloramphenicol shows the highest antibacterial activity against *Salmonella parathypi A*. The synthesized AgNPs exhibits a relatively much higher antibacterial activity against *Salmonella parathypi A*. compared to the RP extract.

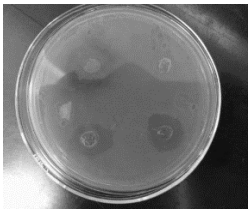


Fig 4. The antibacterial test of AgNPs, RP extract, and Chloramphenicol against *Salmonella parathypi A*.

Table 1. Inhibition zone of each antibacterial agent

Antibacterial agent	Inhibition zone (mm) against *Salmonella parathypi A.* after 20 h incubation
RP extract	1
AgNPs	4
Chloramphenicol (40 ppm)	6
No addition of antibacterial agent	-

4 Conclusions

The rambutan (*Nephelium lappaceum L.*) peel (RP) extract has been used to synthesize AgNPs using aqueous media at room temperature. The analysis of UV-Visible spectra confirms the formation of AgNPs. The SEM analysis shows that the synthesized AgNPs have non-uniform shape and wide distribution of particle size. The synthesized AgNPs have antibacterial activity against *Salmonella parathypi A*. with the inhibition zone of 4 mm, while the inhibition zone of Chloramphenicol against *Salmonella parathypi A*. used as a comparison is 6 mm.

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