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Autoclave-Assisted Synthesis of Silver Nanoparticles using *Metroxylon Sagu* for Antibacterial Application

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Abstract

Background/Objectives: Silver nanoparticles (AgNPs) have been conventionally synthesized through several complicated steps that require careful attention. In this study, a simplified green synthesis method for the production of AgNPs in one pot via autoclaving method was reported. **Methods/ Statistical Analysis:** Different concentrations of Sago solution were mixed with 1 mM $AgNO_3$ and autoclaved at 121 °C for 20 min. The Sago acted as the reducing and capping agent as well as the stabilizer to produce the AgNPs. The synthesized AgNPS were characterized using UV-V is spectrometry, FTIR and EFTEM analysis. The antibacterial assay was also carried out using agar well diffusion method for the investigation of the antibacterial activity of the synthesized AgNPs against Gram positive (*Micrococcus luteus*) and Gram negative (*Escherichia coli*) bacteria. **Findings:** The UV-Vis spectrum for the synthesized AgNPs ranging between 410 – 415 nm, and the shifted peaks of the FTIR spectrum confirmed the presence of the synthesized AgNPs. From the EFTEM images, the AgNPs are spherical in shape and well distributed with average particle sizes of 17.2 ± 6.7 nm. The AgNPs also exhibited great antibacterial activities against both bacteria through the agar well diffusion as clearly observed in the inhibition zones. **Application/Improvements:** Autoclaved and sterilized AgNPs have been synthesized through an environment friendly way and has exhibited great antibacterial activities through the simple and fast method. These AgNPs are suitable for application as promising antibacterial agent in various applications especially in biomedical devices.

Keywords: Antibacterial, Autoclave, Metroxylon Sagu, Silver Nanoparticles

1. Introduction

The development of nanotechnology has attracted much attention lately due to the diversity of their applications. The main characteristic of nanomaterials compared to macro scaled material is their large surface-to-volume ratio. Thus, metallic nanoparticles offer unique antibacterial features due to their high propensity to inhibit bacterial growth in aqueous and solid media[1]. Silver nanoparticles (AgNPs) are of metallic nanoparticles which are largely used as antibacterial agent in the medical field[2,3], textiles[4], cosmetics[5] and waste water treatment[6]. The synthesis of nanoparticles is also being actively pursued through a variety of ways. Conventionally, AgNPs are synthesized through the chemical reduction methods that involve a variety of hazardous chemicals that act as the reducing, capping and stabilizing agents. Besides, the conventional method also involved several steps that require detailed attention including stirring, mixing and monitoring the experiment manually. Hence, various optional methods have been reported to simplify the synthesis methods but still, employing the same concept of chemical reduction. AgNPs have been successfully produced through the incubation[7], microwave irradiation[8], sunlight irradiation[9] and autoclaving methods[10,11].

Other than to simplify the synthesis methods, the use of greener and environmentally friendly materials should also be considered to synthesize AgNPs which at once can eliminate the hazardous wastes. Recently, plant extracts such as the leaves[12], gums[13] and seeds[14] have been the main source of reducing agents during the synthesis of nanoparticles to replace the harmful chemicals. *Metroxylon Sagu* or locally called Sago is a type of starch extracted from Rumbia tree, largely found in Sabah and Sarawak. Currently, sago industry became one of the country's important agricultural export commodities[15]. The extracted Sago is

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processed into powder or 'pearl' form. It is white in colour, tasteless and odourless. Traditionally, the locals consume Sago as their staple food in various forms especially in noodle making. In industries, Sago starch is commercially used as the key material input in the production of paper, plywood and textiles[16]. Sago also can be a best candidate to be used as a reducing agent since starch, a polysaccharide is also a plant extract which has two structurally distinct molecules of amylose and amylopectin. The aldehyde groups from the amylose component will effectively reduce the Ag^+ to Ag.

Thus, in this work, Sago was used as the reducing, capping and stabilizing agent for the green synthesis of AgNPs. Its use effectively eliminated the use of toxic chemicals which are hazardous to the environment. The autoclave-assisted method which is a quick and easy process was also applied for the simplification of the production process in a larger scale.

2. Materials and Methods

The *Metroxylon Sagu* powder was purchased from Dhulau Enterprise, Putatan, Sabah. Analytical grade silver nitrate ($AgNO_3$), nutrient broth, Tryptone Soy Agar (TSA), *Micrococcus luteus* (ATCC 10240) and *Escherichia coli* (ATCC 43888) were used as purchased without further purification throughout the study.

2.1 Synthesis of Silver Nanoparticles

The sago solutions were prepared by adding different (0.1-2.0) volume/weight percentage (w/v %) of sago powder into deionized water. The sago solutions were then mixed with 1mM of $AgNO_3$ solution at the ratio of 1:1.5. The mixtures were autoclaved at 121 °C for 20 minutes for the synthesis of the AgNPs.

2.2 Characterization of Silver Nanoparticles

The synthesized AgNPs were subjected to several characterization processes to prove the presence of the nanoparticles. The Surface Plasmon Resonance (SPR) of the AgNPs was monitored using the UV-Visible spectrophotometer (UV-Vis, Shimadzu UV-2450) at wavelength range of 400 – 800 nm. The functional groups involve in the reduction mechanism of the AgNPs were identified using the Fourier Transform Infrared spectroscopy (FTIR, Thermo Scientific Nicolet iS50) in the wave number range of 400-4000 cm^{-1}. Finally, the size and morphology of the synthesized AgNPs were characterized using the Energy Filtered Transmission Electron Microscopy (EFTEM, Carl Zeiss AG Libra 120).

2.3 Biological Assay of Silver Nanoparticles

The antibacterial activity of the synthesized AgNPs was evaluated through the agar well diffusion method. The Tryptone Soy Agar (TSA) plates were inoculated with approximately 1 x 10^8 CFU/mL of *Micrococcus luteus* and *Escherichia coli*. The agar wells were then punched on the surface with a sterile pore borer to create wells with diameter of 8 mm. Different concentrations of the AgNPs (0 – 100 μL) were pipetted into the well and incubated at 37 °C for 24 h. The zones of inhibition were measured after the incubation period. The results were compared with the control wells without AgNPs.

3. Results and Discussion

3.1 UV-Visible Spectrophotemeter Analysis

Figure 1 shows the UV-Vis spectrums for the synthesized AgNPs with various concentrations of sago. It can be clearly seen that the absorbance peaks increased with increase in the concentration of sago. This is due to the presence of more free Sago which led to increased reduction of the silver with the attendant increase in the production of AgNPs. All the absorbance peaks ranged between 400-415 nm which are the characteristic peak for nanoparticles.

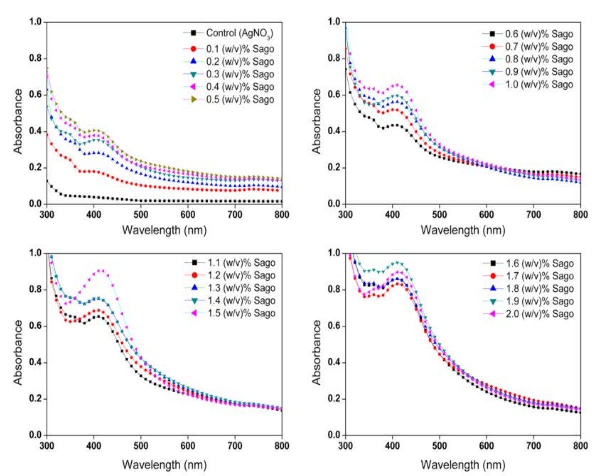

Figure 1. UV-Visible spectrums of the synthesized AgNPs with different sago concentrations.

3.2 FTIR Analysis

The FTIR spectra were recorded for the aqueous sago and the synthesized AgNPs solution to determine the functional groups that were involved in the reduction of Ag^+ to solid Ag. From Figure 2, it was observed that peak 3323 cm^{-1} was shifted to 3328 cm^{-1} due to the high energy stretching of hydroxyl (O-H) groups which were involved in intermolecular hydrogen bonding and resulting in the broad peak. Peak 1654 cm^{-1} also shifted to 1635 cm^{-1} because of the intense vibration of carbonyl (C=O) groups from the aldehyde groups. The C=O is polar and covalently bond and when it stretches, the bond dipole moment changes dramatically resulting in the strong peaks observed in the spectrum. The disappearance of peak 1002 cm^{-1} from the aqueous sago band was due to the reduction of Ag^+, the outcome of C=O and O-H groups oxidation.

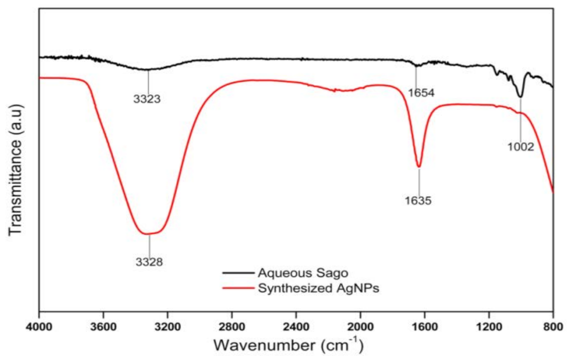

Figure 2. FTIR spectrum of aqueous sago and the synthesized AgNPs.

3.3 EFTEM Analysis

Figure 3 shows the EFTEM image of the synthesized AgNPs at 50 nm scale which showed the spherically-shaped AgNPs with average particle size distributions of 17.2 ± 6.7 nm as measured using the ImageJ software.

3.4 Antibacterial Activity of Synthesized Silver Nanoparticles

From Figure 4, the inhibition zones around the agar well were clearly observed after 24 hours of incubation at 37 °C. The diameters of the inhibition zones increased proportionally with increase in the volumes of AgNPs for both *Micrococcus luteus* and *Escherichia coli* as shown in

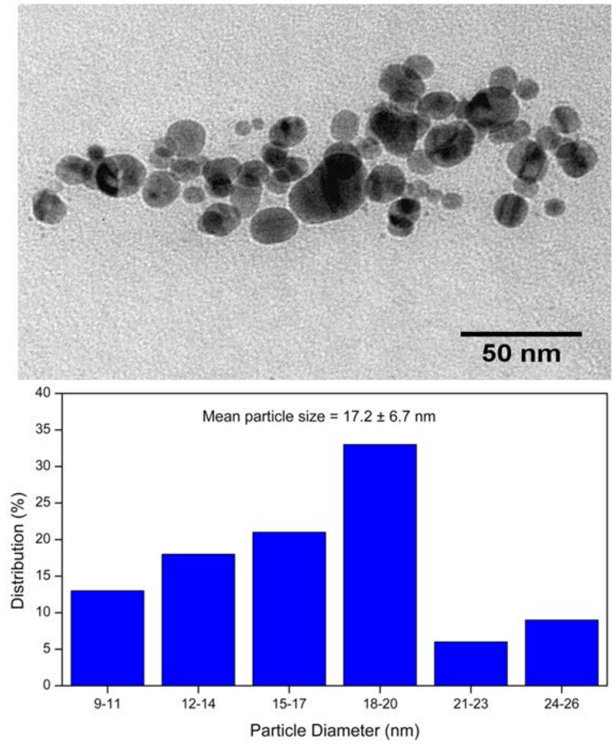

Figure 3. EFTEM image of the synthesized AgNPs and the particle size distribution.

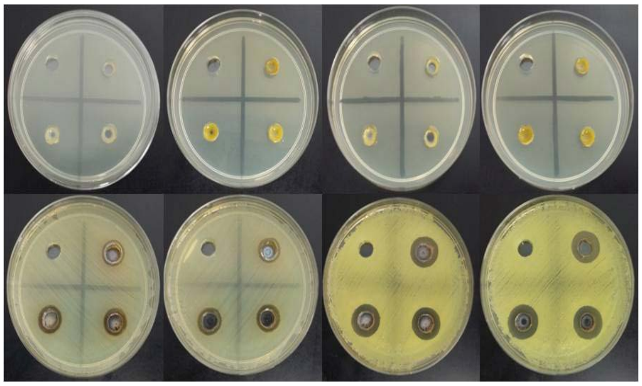

Figure 4. Inhibition zones of *Eschericia Coli* and *Micrococcus Luteus* bacteria with different volumes of AgNPS.

Table 1. Antibacterial activity evaluation of the synthesized AgNPs

Volume of AgNPs solution (μl)	Zone of inhibition diameter (mm)	
	M.luteus	E. coli
0 (Control)	**8.00**	**8.00**
10	16.00	12.73
20	16.17	12.77
40	16.32	13.15
60	16.43	13.32
80	16.73	13.78
100	16.80	14.13

4. Conclusion

In summary, AgNPs were synthesized in a green way for the first time using *Metroxylon sagu* as the reducing, capping and stabilizing agent. The method used is safe without any additional chemical, fast, simple and applicable for large scale production of AgNPs. The AgNPs exhibited great characteristics nanoparticles with absorbance peak of 400 - 415 nm in the UV-Vis spectrum. The EFTEM result confirmed the well distribution of the AgNPs particles with average size of 17 ± 6.7 nm. The investigation of the antimicrobial potential of the synthesized AgNPs showed great effect on the tested Gram positive and Gram negative bacteria. Conclusively, the synthesized autoclaved and sterilized AgNPs can be best used as antibacterial agent in biomedical devices and applications. The International Conference on Fluids and Chemical Engineering (FluidsChE 2017) is the second in series with complete information on the official website[17] and organised by The Center of Excellence for Advanced Research in Fluid Flow (CARIFF)[18]. The publications on products from natural resources, polymer technology, and pharmaceutical technology have been published as a special note in volume 2[19]. The conference host being University Malaysia Pahang[20] is the parent governing body.

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