



GREEN SYNTHESIS OF SILVER NANOPARTICLES USING RHIZHOME EXTRACT OF GALANGAL, *ALPINIA GALANGAL*

(Sintesis Hijau Nanopartikel Perak Menggunakan Ekstrak Rizom Lengkuas, *Alpinia galanga*)

Alyza A. Azmi^{1*} and Norhidayah M. Ahyat²

¹*School of Fundamental Sciences*

²*School of Marine and Environmental Science*

Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

*Corresponding author: alyza.azzura@umt.edu.my

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Abstract

A simple method of synthesizing silver nanoparticles (AgNps) using rhizome extract of galangal, *Alpinia galangal* was presented. Antioxidant contains in galangal served as greener and stable reducing agents in this one-pot synthesis. The antioxidant from galangal was extracted in water at ambient environment and quantitative analysis of antioxidant content was carried out using Total Phenolic Content (TPC) assay. Fourier-Transform Infrared (FTIR) spectroscopy analysis confirmed the presence of $\nu(\text{O-H})$, $\nu(\text{C=C})$ and $\nu(\text{C-O})$ peaks that contributed from polyphenol groups stretching vibrations. The formation of AgNps was tracked by ultraviolet-visible spectrophotometer through the presence of absorption peak at 430 nm, while the morphology and crystallinity of AgNps were determined by Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) analyses. The results from this study prove that antioxidants found in galangal rhizome extract act as effective reducing agent in the synthesis of AgNps.

Keywords: green synthesis, silver nanoparticles, galangal, plant extract, total phenolic content

Abstrak

Kajian ini membentangkan satu kaedah yang mudah dalam sintesis nanopartikel perak menggunakan ekstrak rizom lengkuas, *Alpinia galangal*. Kandungan antioksidan di dalam lengkuas bertindak sebagai agen penurunan yang lebih hijau dan stabil bagi sintesis satu-pot. Antioksidan daripada lengkuas diekstrak menggunakan air pada persekitaran ambien dan analisis kuantitatif bagi kandungan antioksidan telah dijalankan menggunakan ujian jumlah kandungan fenolik. Analisis spektroskopi Fourier Inframerah mengesahkan kehadiran puncak-puncak $\nu(\text{O-H})$, $\nu(\text{C=C})$ dan $\nu(\text{C-O})$ yang disumbangkan oleh getaran regangan kumpulan polifenol. Pembentukan nanopartikel perak dikesan menggunakan spektrofotometer ultralembayung-nampak menerusi kehadiran puncak serapan pada 430 nm, manakala morfologi dan kristaliniti nanopartikel perak ditentukan oleh analisis imbasan elektron mikroskopi dan X-ray pembelauan. Keputusan-keputusan daripada kajian ini membuktikan antioksidan yang dijumpai di dalam ekstrak rizom lengkuas bertindak sebagai agen penurunan yang efektif di dalam sintesis nanopartikel perak.

Kata kunci: sintesis hijau, nanopartikel perak, lengkuas, ekstrak tumbuhan, jumlah kandungan fenolik

Introduction

Nowadays, the investigation on silver nanoparticles, AgNps becomes a great interest as it has contributes wide applications in diverse fields for instance; cancer therapeutic [1], catalysis [2], dye degradation for water treatment [3] and textile industry [4]. Numerous of routes were formulated in past studies for synthesizing AgNps such as thermal decomposition [5], γ -irradiation [6], laser ablation [7] and electron irradiation [8]. However, the uses of

hazardous reducing agent in the previous routes such as sodium borohydride, NaBH_4 , which may enhance environmental pollution, thus limiting the Ag-Nps applications especially in clinical field. Due to this reason, a green approach for synthesizing AgNps has been conducted by substituting previous reducing agent with natural bioactive compounds. Green technique becomes a potent aspect for current nanotechnology research because it is environmental friendly, safe, cost effective and required renewable reducing agent.

Some natural bioactive compounds, which are extracted from yeasts, enzymes, fungi, bacteria and plant are appropriate to reduce Ag^+ ion, from (I) to (0) oxidation state. This is due to the abundances of hydroxyl and amino groups that present in these natural resources. Among of these sources, plant extracts would be the most preferable reducing agent for synthesizing AgNps as it reduces synthesis time consumption as well as considering the production scale is higher compared to other natural resources. Production of AgNps from plant extracts for example; *Curcuma longa* [9], *Hibiscus rosasinensis* [10], *Punicagranatum* [11] and aquatic weeds [12] have been reported literally. In the present study, antioxidant extracted from galangal rhizome, *Alpinia galanga* has been used to synthesize Ag-Nps. The abundances of phenolic groups present in the antioxidants extract is expected to reduce silver ions to silver metal, thus stabilizing the prepared AgNps from being aggregated.

Materials and Methods

Galangal rhizome, *Alpinia galanga* was purchased from the local market in Kuala Terengganu, Malaysia. Silver nitrate, AgNO_3 (0.1 M), Folin-Ciocalteu reagent, gallic acid, sodium carbonate, NaCO_3 (7.5 %), were purchased from Sigma Aldrich and used without purification. Deionized water was used throughout the reaction.

Preparation of Galangal Rhizome Extract

Galangal rhizome was washed to remove impurities, dried at ambient temperature, powdered using IKA A11 basic grinder and sieved to get uniform sizes powder (approximately 355 μm) before the extraction begin. Rhizome extract was prepared by adding 0.1 g of rhizome powder in 25 ml of deionized water. The mixture was then stirred vigorously for 4 hours in room temperature.

Determination of Antioxidant in Galangal Rhizome

Vibration of functional groups in antioxidant of galangal rhizome was characterized via Fourier Transform Infra Red, FTIR (Perkin Elmer 100 spectroscopy) with frequency range 4000 to 450 cm^{-1} with transmission mode using KBr support. Total phenolic content of rhizome extract was determined by total phenolic content, TPC assay. Water extract of galangal rhizome was prepared (0.1 g of rhizome powder in 25 ml of deionized water), mixed with Folin-Ciocalteu (FC) reagent (1 ml), and then stirred for 3 minutes. Sodium carbonate (4 ml, 7.5 %) was added into the prepared solution and incubated in dark for 2 hours. Absorption reading of phenolic content was observed ($\lambda = 765 \text{ nm}$). The concentration of phenolic compound in the rhizome extract was expressed as gallic equivalent (GAE/100 g).

Green Synthesis of Silver Nanoparticles

Rhizome extract (0.1 g rhizome powder in 25 ml deionized water) was added into silver nitrate, AgNO_3 solution (30 ml, 0.01 M). The reaction was left for 48 hours in one-pot under room temperature in dark to avoid any photochemical reduction. Effect of time (96 and 144 hours of reactions) and concentration of AgNO_3 solution (0.001 M and 0.1 M) were observed.

Characterization of Silver Nanoparticles

Surface plasmon resonance, SPR absorption of prepared AgNps was observed using UV-Vis analysis (Shimadzu UV-Vis 1601 series) in 380 – 450 nm range. Measurement on crystallinity of AgNps was determined using X-ray diffractometer, XRD (Rigaku MiniFlex II) instrument equipped with Ni-filtered $\text{Cu K}\alpha$ radiation as the X-ray source. It has been set up in continuous mode with diverging and receiving slit 0.0° , scans speed $0.005^\circ/\text{second}$ with scan range 10° to 90° . Surface and morphology of AgNps was determined using Scanning Electron Microscope, SEM. Samples were mounted on stainless steel sample stub and pre-coated with thin layer of gold. The SEM was operated at 16 kV with various magnifications.

Results and Discussion

FTIR Spectroscopy of Galangal Rhizome

FTIR spectroscopic study was carried out to investigate the possible vibration of functional groups in rhizome powder. FTIR spectrum (Figure 1) exhibited broad peak at 1638.6 cm^{-1} and 1424.1 cm^{-1} , which was assigned to the presence of the aromatic group in the water extract. Stretching of hydroxyl (-OH) group was observed at peak 3400.8 cm^{-1} . Vibration peak at 1053.5 cm^{-1} represent carbonyl (C=O) stretch. It was observed that vibration of sp^3 alkyl group at peak 2930.1 cm^{-1} and supported through the vibration of long-chain band at peak 772.6 cm^{-1} , 616.8 cm^{-1} and 526.4 cm^{-1} . From these vibrations, long-chain band of the alkyl group was predicted in the galangal rhizome. Table 1 below showed list of possible vibration of functional groups in rhizome powder.

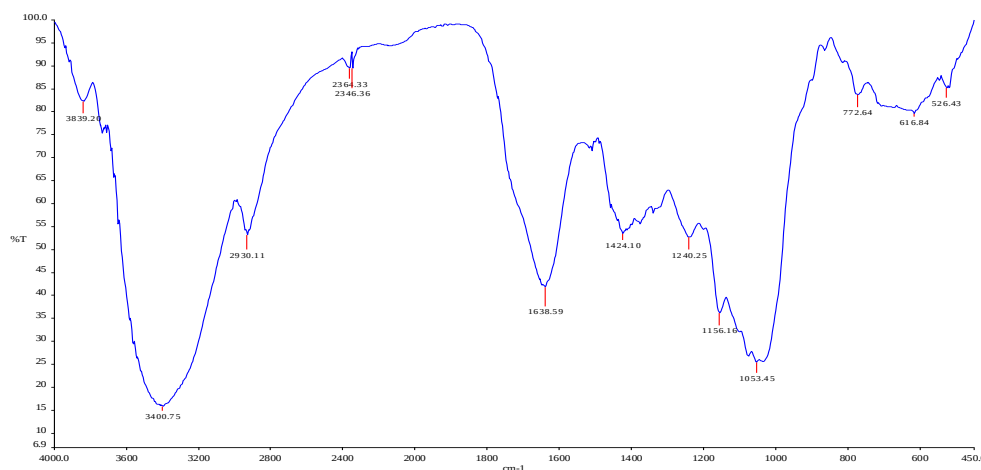


Figure 1. FTIR spectrum of rhizome powder

Table 1. List of functional group vibrations in rhizome powder.

Functional group	Type of vibration	Frequency (cm^{-1})
O – H	Phenols stretch	3400.8
C = C	Aromatic stretch	1638.6 and 1424.1
C – O	Carbonyl stretch	1053.5
C – H	Alkyl stretch	2930.1
	Long chain band	772.6, 616.8 and 526.4

Total Phenolic Content of Galangal Rhizome

Absorption reading of gallic acids and rhizome extract (Table 2) was taken at 765 nm. From the absorption reading, the calibration equation was determined as $y = 0.0019x + 0.0012$ (y represent the absorption of phenolic groups at 765 nm, x represent the concentration in $\mu\text{g/ml}$). From the equation, the concentration of rhizome extract was 13.94 ppm and the calculated total phenolic compound presented in rhizome extract was 0.02492 GAE/100 g.

Green Synthesis of Silver Nanoparticles

Formation of AgNps was detected by observing the colour changes of prepared solutions (Figure 2), which was due to electron transitions of surface plasmon resonance in the solutions. Manipulation on time of reactions (48, 96 and 144 hours) has increased the colour intensity of Ag-Nps colloidal solution from colourless to light brown (48

hours), light brown to brown (96 hours), and brown to dark brown (144 hours). Further characterization on reduction of Ag (I) using rhizome extract was determined using spectroscopic techniques for analyzing the formation of Ag-Nps.

Table 2. Absorption reading of gallic acids and rhizome extract.

Concentration ($\mu\text{g/ml}$)	Absorption at 765 nm, A
10	0.0013
30	0.0206
40	0.0601
50	0.0989
Rhizome extract	0.0277

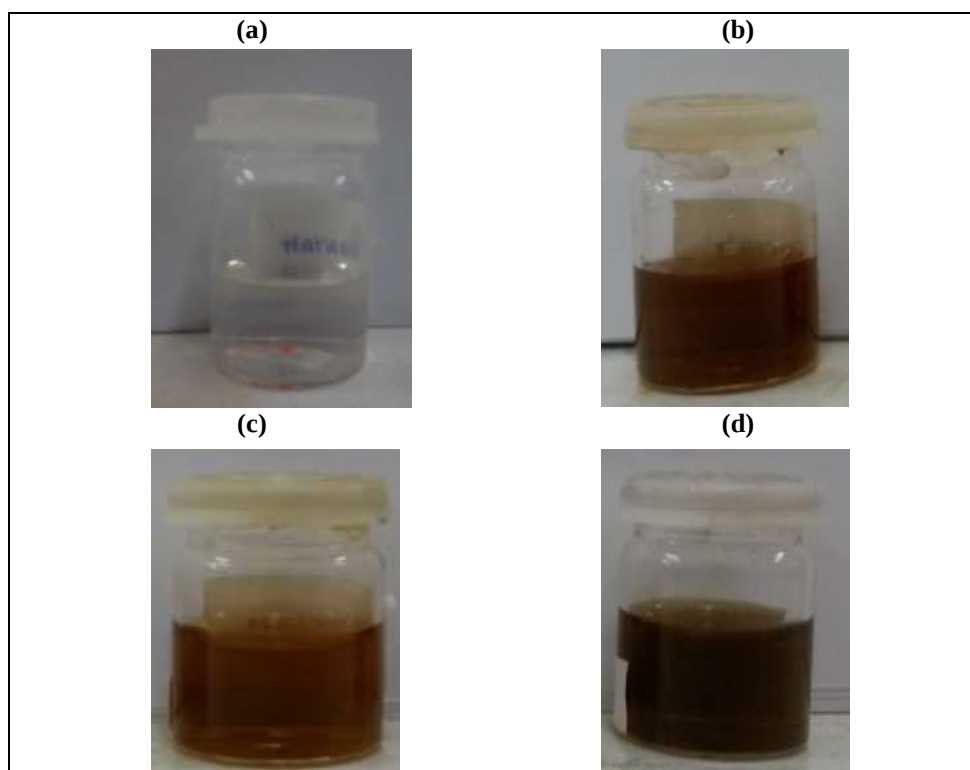


Figure 2. a) AgNO_3 solution before addition of water extract. AgNO_3 solution after addition of water extract at b) 48 hours, c) 96 hours and d) 144 hours.

UV-Vis Spectroscopy Analysis of Silver Nanoparticles

Previous study stated that the formation of SPR band was influenced by several conditions of Ag-Nps such as size, morphology and composition of prepared Ag-Nps [9]. SPR bands of prepared Ag-Nps were determined in wavelength range 300 to 800 nm, respectively. In this study, different concentration of AgNO_3 (Figure 3) used has affected the absorption of SPR bands. From the spectra, it clearly proved that Ag-Nps with 0.01 M of AgNO_3 showed the highest intensity of absorbance, when compared to the concentration of 0.001 and 0.1 M. Besides, the

stability of Ag-Nps with 0.01 M of AgNO_3 was tested by increasing time of reaction from 48, 96 and 144 hours (Figure 4). The absorption peak showed that there was slightly shifted around 431 to 438 nm.

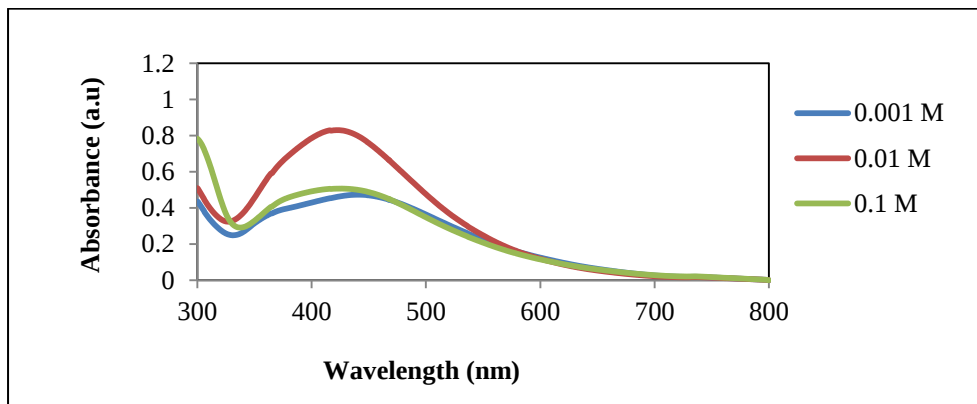


Figure 3. UV-Vis spectra of Ag-Nps in different concentrations after 48 hours.

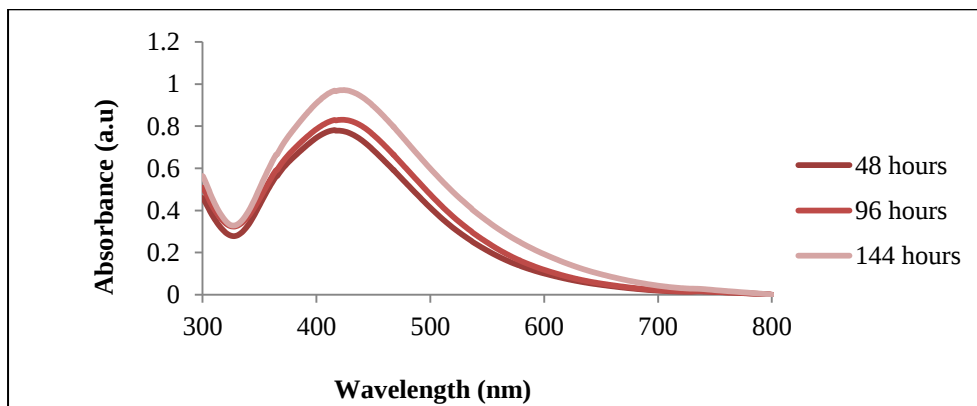


Figure 4. UV-Vis spectra of Ag-Nps in different concentrations after 48 hours

X-Ray Diffraction Analysis of Silver Nanoparticles

Powder x-ray diffraction pattern of the prepared colloidal Ag-Nps was shown in Figure 5. XRD patterns at peaks 2θ of 39.28° , 43.04° , 60.54° and 73.14° can be attributed that the structured of Ag-Nps was face centred cubic [13], which was corresponding to (111), (200), (220) and (311). From all samples, it was observed that the main crystalline phase was silver and there are two sharp peaks at peaks 2θ around 45° to 50° , which were belong to the organic compound which was from the antioxidant of water extract [14].

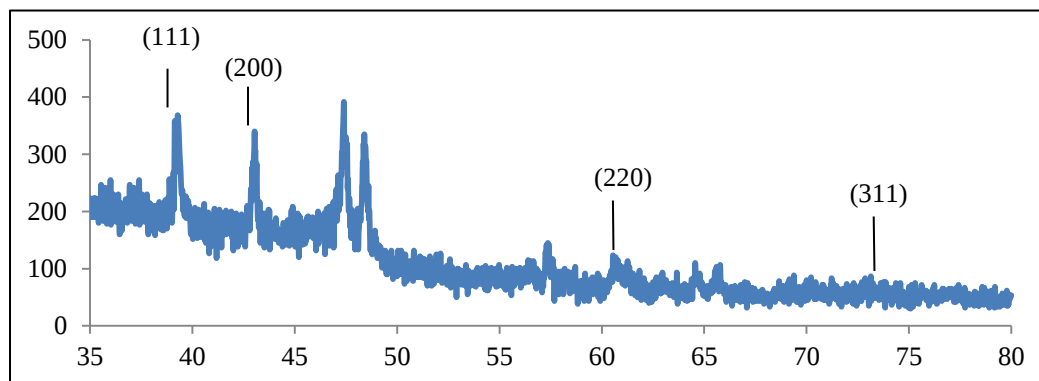


Figure 5. X-ray diffraction pattern of prepared Ag-Nps.

Surface and Morphology of Silver Nanoparticles

Scanning electron microscope showed the surface and morphology study of the Ag-Nps. Figure 6 depict the preliminary data of SEM photograph for the one of Ag-Nps sample prepared and the spherical morphology was detected.

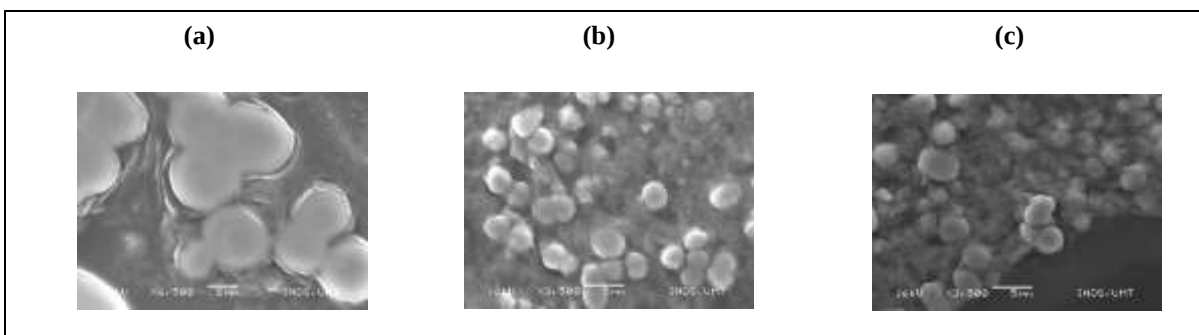


Figure 6. SEM photograph of the prepared Ag-Nps with the 0.01 M of initial concentration of AgNO_3 (Magnificent of (a) x6500, (b) x3500 and (c) x3500)

Conclusion

In conclusion, the antioxidant of galangal rhizome extract was successfully to synthesize and stabilized prepared Ag-Nps colloidal solution at room temperature in a certain period. The prepared Ag-Nps were dispersed in spherical shape and they are crystalline in nature with FCC structures. From the results, it was proved that galangal rhizome would become good reducing agents for preparing Ag-Nps. Prepared Ag-Nps was expected to show antimicrobial properties towards human pathogenic bacteria, which is it can substitute the conventional antimicrobial agents with the 'green' Ag-Nps prepared.

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