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RESEARCH ARTICLE

Green synthesis of silver nanoparticles from *Alpinia galanga* extract with microwave irradiation and antibacterial activity against *Escherichia coli*

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Abstract

Background: This study deals with the rapid green synthesis of silver nanoparticles (Ag NPs) by microwave irradiation using water extract of *Alpinia galanga* as a reducing agent. **Methods:** The effect of concentrations of water extract and silver nitrate aqueous solution (1 mM) on the synthesis was investigated using the ratios 1:1, 1:5, 1:10, and 1:20. The formation of Ag NPs was observed after being microwaved for 30, 60, 90, 120, 150, and 180 seconds. **Results:** The best Ag NPs were produced from the ratio of 1:10 after 180 s irradiation, confirmed by the optimum absorbance at 450 nm using a UV-visible spectrophotometer. Transmission electron microscopy analysis showed that the size of the Ag NPs was 50 nm. Their antibacterial activity was determined using Resazurin microtiter assay against *Escherichia coli*, and the results showed that it inhibited 94.4% of *E. coli*. It was significantly different ($p < 0.05$) with the inhibition of extract and Amoxsan, which inhibited 71.1% and 85.8% of *E. coli*, respectively. **Conclusion:** The use of microwave irradiation on the synthesis of silver nanoparticles using *Alpinia galanga* extract as a reducing agent appears to be rapid and eco-friendly.

Introduction

Nanotechnology is a rapidly emerging field, with applications across science and technology, concentrating on the synthesis and manipulation of particle structures ranging in size from about 1 to 100 nm (Alabdallah & Hasan, 2021; Rodríguez-Félix *et al.*, 2021). Metal nanoparticles, such as gold, silver, and platinum, offer a plethora of potential medicinal and electrical uses. The nanoscale size and surface-to-volume ratio of these materials enhance their physical and chemical characteristics (Tailor *et al.*, 2020; Patil *et al.*, 2021). Nanosilver, which is a commercialised nanomaterial, has been used as an antimicrobial agent and has been incorporated into textiles, medical devices, electronics, and water purification systems, as well as topical creams and antiseptic sprays, among other applications (Asghar *et al.*, 2017; Elshafei *et al.*, 2021; Jaast & Grewal, 2021). They are often manufactured and stabilised using physical and chemical processes, but their applicability is limited due to the presence of hazardous compounds. As a result, it is critical to creating non-toxic and environmentally benign methods for synthesising nanoparticles, particularly Ag NPs.

Green Ag NPs production is both environmentally friendly and cost-effective. Because it does not require high pressure or harmful ingredients, it can be easily scaled up to large-scale synthesis. Many studies have been conducted on the utilisation of fungus, bacteria, and plant extracts. The bio-synthetic method that uses plant extracts as reducing agents has evolved as a simple and fast technology because it gives a one-step approach to biosynthesis (Alabdallah & Hasan, 2021; Jalab *et al.*, 2021; Oves *et al.*, 2021). Plant extracts are widely accessible, easily handled, and contain a diverse range of metabolites that aid in the reduction process. The size, shape, and antibacterial activity of nanoparticles produced via plant extract, however, depends on the type and concentration of phytochemicals present in the plant, the synthesis temperature, and the reaction time. This paper describes an environmentally acceptable and efficient method for biosynthesis of Ag NPs using *Alpinia galanga* extract and microwave irradiation. It also investigates the influence of reaction time and the silver nitrate/extract ratio. The extract and produced Ag NPs antibacterial activity was determined against *Escherichia coli.*

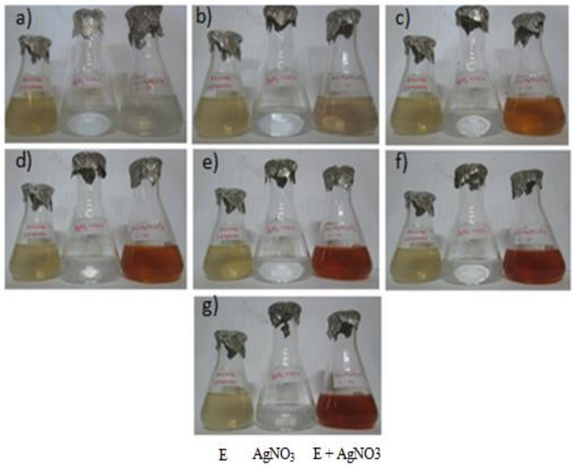
Methods

Alpinia galanga rhizomes were washed thoroughly with sterilised distilled water and then cut into small pieces. 50 g samples were boiled with 150 mL distilled water for 5 minutes and then ultra-sonicated for 30 minutes. The extract was allowed to cool at room temperature, filtered using Wattman filter paper No. 1, and then concentrated using a rotary evaporator.

For the synthesis of Ag NPs, various ratios of extract and 1 mM aqueous solution of silver nitrate were prepared, i.e. 1:1, 1:5, 1:10, and 1:20, and then put into the microwave (Samsung Model ME109F) for different ranges of time (30, 60, 90, 120, 150, and 180 seconds). The formation and size of Ag NPs were determined using UV-Visible, FT-IR, and Transmission electron microscopy (TEM). Anti-bacterial activity against *E. coli* was determined for extract and nanosilver using Resazurin microtiter assay with Amoxsan as a positive control.

Results

The formation of AgNPs was monitored by a color change in the aqueous solution. Figure 1 shows the color changes from time to time for the solution with 1:10 of the silver nitrate/extract ratio as representative. To observe the effect of exposure time of microwave irradiation on the formation of AgNPs, aliquots of the reaction solution were taken periodically and subjected to UV-vis spectroscopy measurement. UV-vis spectrophotometer was used to ascertain the formation of AgNPs at 300 nm to 600 nm. The absorbance intensity of the reaction mixture increases exponentially with irradiation exposure time, as depicted in Figure 2. Of all products from different variables, silver nitrate/extract ratio of 1:10 with 180s irradiation was the best condition in microwave assisted-synthesise of AgNPs using *Alpinia galanga* extract.



Note: 1 mM AgNO3 without extract, and 1 mM AgNO3 with extract after (a) 0 s, (b) 30s, (c) 60 s, (d) 90 s, (e) 120 s, (f) 150, and (g) 180 s of reaction time in microwave.

Figure 1: Digital photographs of extract

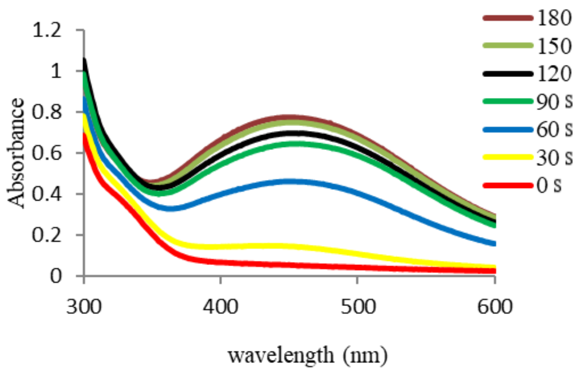


Figure 2: UV–vis absorption spectrum of Ag NPs (1:10) for different reaction times

FTIR spectra indicate strong absorption bands at 3208, 2924, 1645, and 1045 cm-1. TEM image of the AgNPs was shown in Figure 3, which indicated that morphologically, the AgNPs are spherical and rod-like with an average size of 50 nm. Furthermore, inhibition data of AgNPs against E. coli showed that it has higher antibacterial activity than Amoxsan and the water extract. Colour changes of the resazurin test were presented in Figure 4.

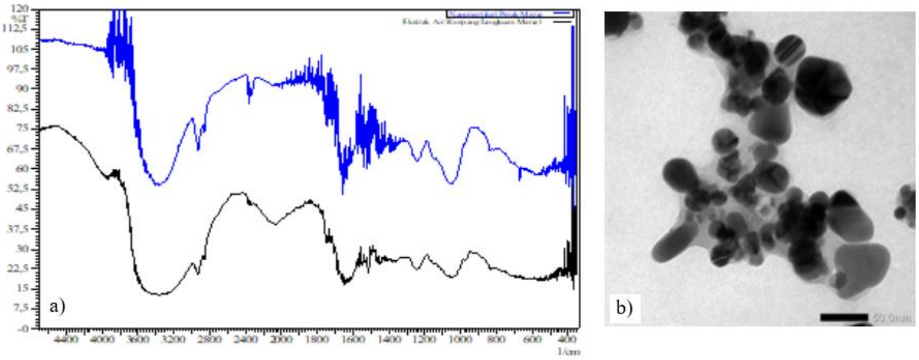


Figure 3: FTIR spectra (a) and TEM photograph (b) for silver nanoparticle (ratio of 1:10;180s reaction time)

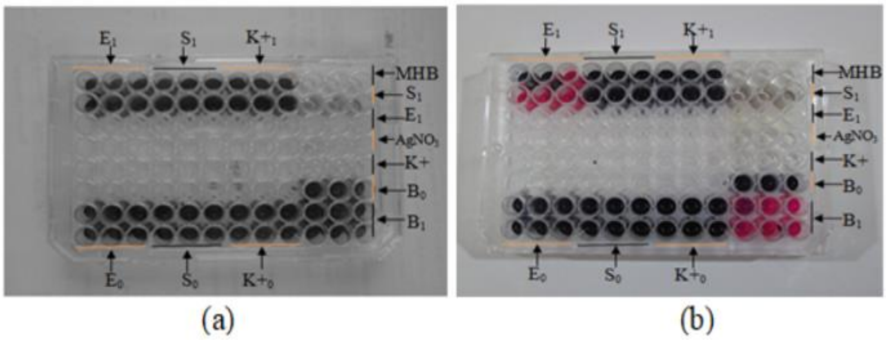


Figure 4: Antibacterial test (a) before incubation, (b) after 18 h incubation

Discussion

The use of plants as the production assembly of Ag NPs has drawn attention because of its rapid, eco-friendly, non-pathogenic, and providing a single-step technique for the biosynthetic processes. On mixing the extract with 1 mM AgNO3 solution, the colour of the solution changed from pale yellow to yellowish-brown, and the final colour deepened with an increase in reaction time (Figure 1) due to the Surface Plasmon Resonance (SPR) phenomenon (Ider *et al.*, 2017; Kaman & Dutta, 2019). The formation of silver nano was confirmed using UV-Vis spectral analysis. In agreement with previously published reports, we observed an increase in the reduction rate of silver ions (Ag+) by increasing the concentration of AgNO3 (Kaman & Dutta, 2019; Rodríguez-Félix *et al*., 2021). The increasing intensity of absorbance peak indicates the increasing formation of AgNPs, and a sharp peak indicates the formation of nearly monodispersed nanoparticles. The finding of this study showed that the characteristic surface plasmon resonance band occurred in the range of 410-500 nm for all ratios used. The best spectra were produced by the ratio of 1:20 from 180s irradiation, confirmed by the optimum absorbance at 450 nm (Figure 2).

Further characterisation and antibacterial activity were only carried out for AgNPs produced from the ratio of 1:10 with 180s reaction time. FTIR spectra of extract and AgNPs (Figure 3a) show an intense broad spectrum at 3208 cm-1, characteristic of the hydroxyl functional group in alcohols and phenolic compounds. A band at 2924 cm-1 corresponds to the aliphatic −CH stretching, while a band at 1645 cm-1 indicates CO- (NH) group. The hydroxyl group of alcohols and carbonyl groups from the amino acid residues have a strong affinity to bind metals; therefore, they can act as an encapsulating agent and thus protect the nanoparticles from agglomeration (Kanniah *et al.,* 2021).

Transmission electron microscopy was used to provide further insight into the size, shape, and morphology of the synthesised AgNPs. The TEM image presented in Figure 3 (b) reveals that the average diameter of the synthesised AgNPs was 50 nm. The AgNPs were surrounded by a faint thin layer of other material, the capping organic material from the plant extract, preventing AgNPs aggregation, providing stability, and increasing their lifetime (Sidhu & Nehra, 2019).

The microplates of the antibacterial activity test are given in Figure 4. Inhibition was recorded at 600 nm. Ag NPs exhibited good antibacterial activity against *Escherichia coli.* It showed higher inhibition (94.419±0.610%) than Amoxan 1000 ug/mL (85.825±0.589%) and extract (71.093±0.254%). Multiple processes could be involved in the mechanism of *Escherichia coli* inactivation using Ag-NPs, including indirect generation of reactive oxygen species or direct interaction of silver with proteins and lipids in the cell wall and proteins in the cytoplasmic membrane that involved in transport and respiration metabolism (Chamakura *et al.,* 2011; Tang & Zheng, 2018).

Conclusion

The authors have reported the synthesis of Ag NPs by using a water extract of *Alpinia galangal,* which provides a simple and efficient way to synthesise nanomaterials. Ag NPs prepared in this process are quite fast-acting and low cost. The characterisation of Ag^+ ions exposed to these plant extracts by UV-vis and TEM techniques confirms silver ions' reduction to Ag NPs. Higher bactericidal activity than Amoxsan indicated that biological synthesised Ag NPs could be of immense use in the medical field for their efficient antimicrobial function.

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