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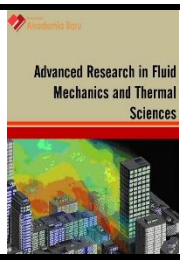

Microwave-Assisted Green Synthesis of Ag Nanoparticles using Leaves of Melia Dubia (Neem) and its Antibacterial Activities

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ABSTRACT

The biosynthesis methods of nanosilver are of interest due to its low cost and environmental friendliness compared with chemical and physical methods. This study will conduct a green synthesis of nanosilver by decreasing the $AgNO_3$ using leaf extract with the presence of stabilized microwave irradiation and collagen. *Melia dubia* leaves, and hydrolyzed fish scale collagen were utilized as reducing and stabilizing agents, respectively. Ag nanoparticles (AgNPs) were synthesized and characterized via Uv-Vis spectroscopy, scanning electron microscope (SEM) that attached with energy-dispersive X-ray, X-ray diffraction (XRD), and antibacterial activities. Results showed that the spherical shape of AgNPs was formed with particle size ranging from 72 nm and 100 nm. UV–vis analysis revealed that the absorbance peak was observed at 446 nm, which corresponded to AgNPs. XRD analysis confirmed the natural crystalline of AgNPs with a particle size of 84.8 nm. The antimicrobial analysis was conducted using *Staphylococcus aureus* and *Escherichia coli* as Gram-positive and -negative bacteria, respectively. Results showed the inhibition zone against both bacteria, which indicated excellent antimicrobial properties of the samples.

1. Introduction

Interest in nanomaterials has significantly increased in recent years as considerable numbers of nanomaterials have been produced. Despite the importance of nanomaterials, the need for environmentally-friendly materials are a crucial aspect of these materials. Researchers are required to find ways to produce non-toxic and environmentally-friendly nanomaterials. Researchers synthesized materials via green chemistry (green synthesis), which resulted in materials with good

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and non-toxic qualities that considered to be harmless to the environment, to solve this problem [1-2].

Metal nanomaterials have become a topic of interest in the research field due to their individual optical and electrical features. The chemical and physical characteristics of metal nanoparticles (NPs) have attracted the attention of researchers to control their sizes and scales in obtaining the required chemical and physical properties for many applications [3-4]. AgNP is an important metal NP because of its unique optical and electronic features and its various applications, such as pharmaceutical, biomedical, sensor technology, and catalysis tics [5]. Several techniques, such as photochemical, microemulsion, chemical, microwave, and electrochemical ones, have been documented to synthesize AgNPs. Many of these techniques are used to synthesize AgNPs, including the use of chemical materials. Most methods used to synthesize AgNPs by utilizing chemical materials, which may cause toxicity to AgNPs, thereby polluting the environment. Researchers have synthesized AgNPs using biological materials that are environmentally friendly and cheap; one of these methods is known as green synthesis, which uses extract plants as the reducing agents to decrease the AgNPs [6-8].

Despite the properties possessed by biological processing methods, these methods are slower than chemical methods. Chemical methods are integrated with biological ones to overcome the disadvantage mentioned above. The radiation of the microwave will cause a prompt and constant heat reaction onto the medium and, thus, offer similar circumstances to the nucleation and growth, thereby causing monodispersed within a short period of time [9]. *Melia dubia* leaves (India) were used to synthesize nanosilver with a size of 7.3 nm via green synthesis; these leaves showed a good anticancer activity [10] because additional *M. dubia* (India) leaf extract has been used to synthesize n-ZnO [11]. However, synthesized AgNPs from *M. dubia* (neem) leaf for antimicrobial activity were not reported.

Antibacterial activities for AgNPs were investigated against a wide range of pathogens, such as *Escherichia coli*, *Staphylococcus aureus* [12], *S. typhi*, *Pseudomonas aeruginosa* [13-14] and *Acinetobacter baumannii* [15]. These pathogens have shown promising activities.

In the present study, microwave-assisted green synthesis was used to manufacture AgNPs using $AgNO_3$ as starting material and *M. dubia* leaves and collagen as reducing and stabilizing agents, respectively, which are antimicrobial agents. Figure 1 shows the fresh and dried leaves of *M. dubia*. The antibacterial properties of AgNPs were investigated among *E. coli* and *S. aureus* as Gram-negative and -positive bacteria, respectively. Low-cost and environmentally-friendly NPs have a range of sizes from 70 nm to 100 nm, which are highly effective against bacteria, have been successfully synthesized. Second paragraph starts here. A nanofluid can be produced by dispersing metallic or non-metallic nanoparticles or nanofibers with a typical size of less than 100 nm in a base liquid.

Fig. 1. Fresh and dried leaves of *Melia dubia* (neem)

2. Materials and Methods

2.1 Materials

$AgNO_3$ was purchased from Bendosen. *M. dubia* (neem) leaves were collected locally from Perak, Malaysia. Collagen from the fish scales was produced in the chemistry laboratory of the Chemistry Department in UPSI, Perak, Malaysia. Nutrient agar and broth were purchased from Merck.

2.2 Methods

2.2.1 M. dubia (neem) leaf preparation

Fresh *M. dubia* (neem) leaves were collected from Perak. The leaves were washed numerous times to remove all dust and fungi and dried using the sun-dried method for one week to remove moisture completely. Dried leaves were converted to powder form through crushing. A total of 10 g dried powder was extracted with distilled water in a volume of 100 ml in a 250 ml conical flask. Afterward, this powder was heated for 10 minutes, cooled, and filtered to obtain a crude extract and later incubated at 4 °C.

2.2.2 Microwave-assisted green synthesis of AgNPs

A total of 1 mM of $AgNO_3$ was dissolved in distilled water with a volume of 90 ml and transferred to a 250 ml conical flask. An additional 10 ml of crude extract was poured to the conical flask, and the mixture was stirred well. Then, collagen (FsCol) of 0.1 g was added, and the mixture was stirred well [6]. A conical flask was put into a microwave oven (ME711K [800]), which operates at a frequency of 2450 MHz, power of 800 W, and underwent microwave irradiation for 3 min. The development of the AgNP was analyzed using UV–vis spectroscopy. The solution of the AgNP was centrifuged at the rate of 14000 rmpfor 10 min. The part that is supernatant was discarded, and the part of NP was sterilized in distilled water. The process as mentioned earlier was conducted repetitively for three times to eradicate any unused biomaterial and impurity. Dry particles were obtained for the purified sample through freeze-drying [9].

2.3 Characterization of AgNP

The absorbance between 200 nm and 800 nm is done using the UV–vis spectroscopy or Agilent Cary 60 UV Spectrophotometer. Energy-dispersive X-ray spectroscopy (EDX) by Bruker X Flash 6110, scanning electron microscopy (SEM) by Nova NanoSEM 450, scanning transmission electron microscopy (STEM), and X-Ray diffraction (XRD) were respectively performed using HITACHI SU8020 and Bruker D2 Phaser, and TEM was done by using Philips CM300.

2.4 Antibacterial Activity

Antibacterial investigations were done for Gram-negative *E. coli* and Gram-positive *S. aureus* by employing the method of paper disk diffusion. Nutrient agar media were used to cultivate bacteria, and 10 µg of AgNPs was saturated in 1 ml of distilled water and checked against bacteria. Plant extracts were also examined. A total of 10, 20, 40, 60, and 80µM/ml of AgNPs were dissolved in distilled water and poured on a 6 mm disk filter [16]. The inhibition zones were measured after 24 h of incubation. The magnitude of antibacterial effects against Gram-negative *E. coli* and Gram-positive *S. aureus* was determined based on the inhibition zone measured in the disk diffusion test.

3. Results

3.1 UV-Vis Spectroscopy

UV–vis spectroscopy tool was used to conform to AgNP stability and formation in the solution. The extinction spectra of AgNPs are depicted in Figure 2. The absorption band of the synthesized AgNPs was observed at 446 nm. Many studies have documented the absorbance of AgNPs; the peak of surface plasmon for AgNPs with asize of <100 nm is located between 400–450 nm [17]. This study observes the surface plasmon peak at 460 nm, which shows that the AgNPs size was less than 100 nm. Other methods have confirmed of a similar phenomenon.

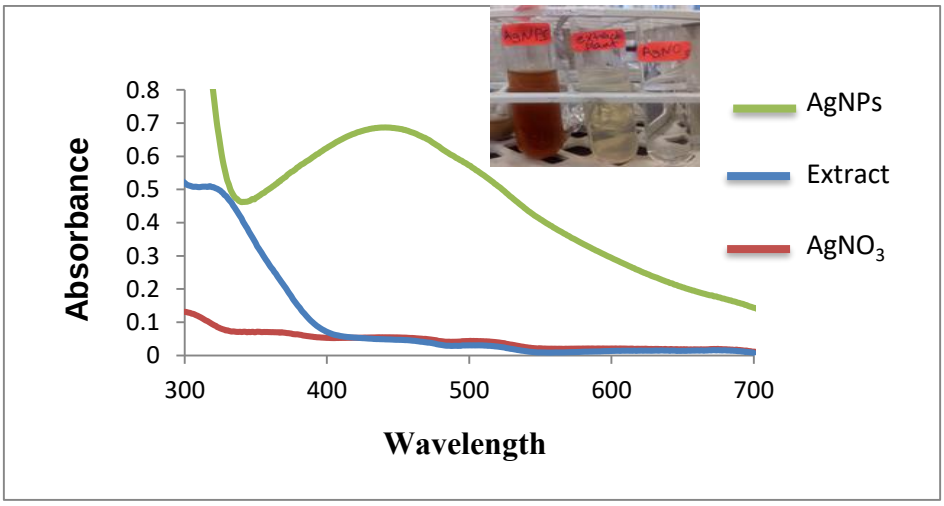


Fig. 2. Uv-vis spectroscopy of AgNPs, extract, and $AgNO_3$

3.2 SEM (Scanning Electron Microscope)

SEM is used to categorize the parts of the synthesized AgNPs. The SEM image of SEM shows the constant distribution of AgNPs. It is observed that the shape of AgNPs is spherical with smooth morphology. Figure 3 shows the SEM image, the magnification of the SEM image was at 10000x. The average size of the particles was approximately 84.8nm which have been confirmed by using TEM and XRD. The similar shape for the silver nanoparticles by using microwave-assisted have been reported in the literature [18].

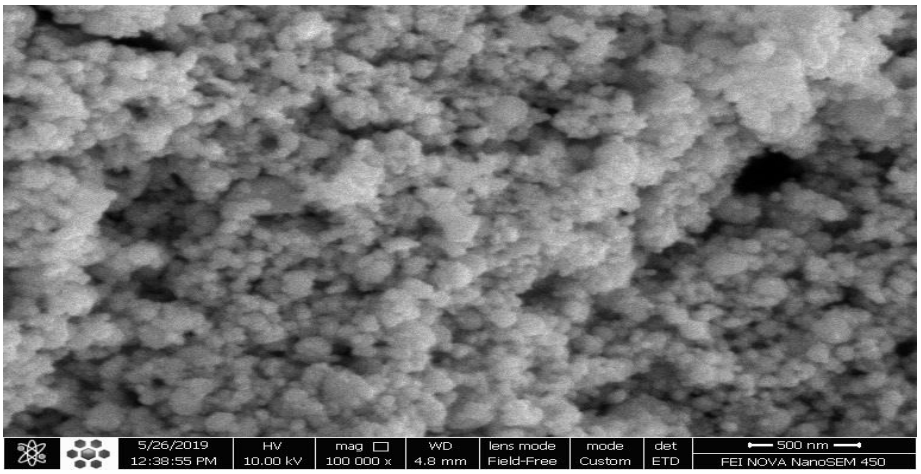


Fig. 3. Surface morphology of synthesized AgNPs in SEM

3.3 EDX (Energy-Dispersive X-ray Spectroscopy)

The presence of Ag as a single NP is confirmed with the use of EDX. The EDX profile indicated the presence of Ag in approximately 3 keV, which was referred to as Nanocrystalline Ag. The peaks of the oxygen and carbon were also observed due to their natural existence in the plant extracts. This result indicates the presence of AgNP surrounded by carbon and oxygen [19]. Figure 4 shows the presence of Ag along with carbon and oxygen and element distribution mapping.

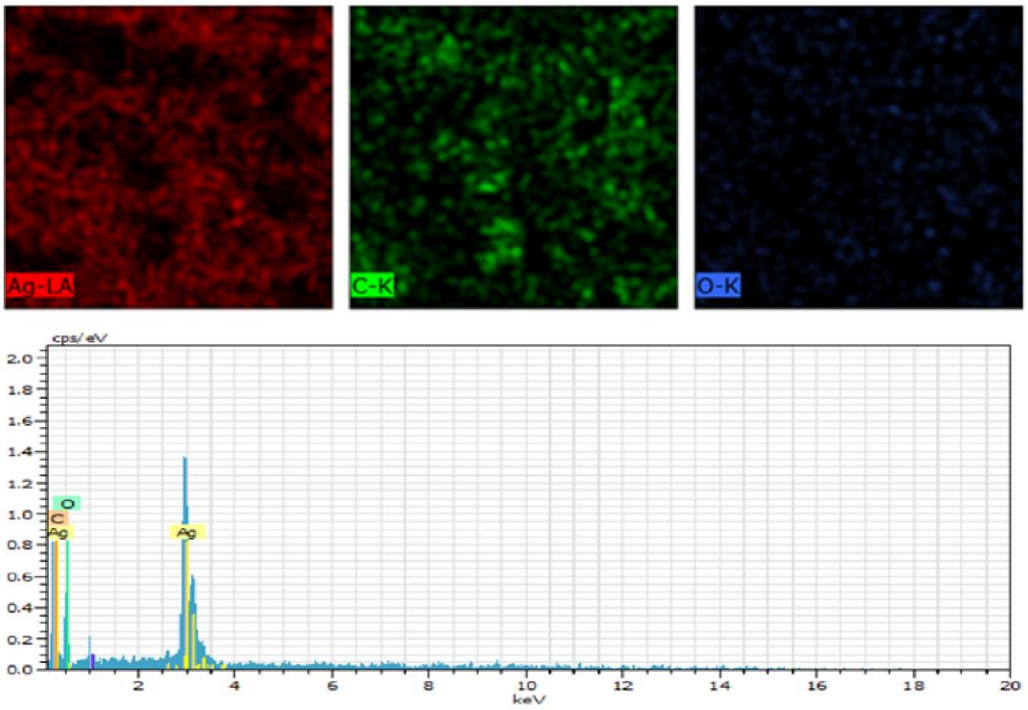


Fig. 4. Mapping distribution of elements in EDX

3.4 XRD (X-ray Powder Diffraction)

XRD was used to confirm the size and natural crystalline of AgNPs, as presented in Figure 5. The AgNPs XRD spectrum exhibited four peaks at the 2θ values of 38.48°, 44.1°, 64.38°, and 77.34°. XRD data were analyzed using the Match 3 software. The analysis report shows the cubic shape for synthesized AgNPs; the report reference belongs to 96-500-0219. The size of the AgNPs particle was determined based on the following equation.

$$d = 0.9\lambda / \beta\cos\theta, \quad (1)$$

d refers to the mean diameter of NPs, *λ* refers to the wavelength of the X-ray radiation source, and *β* refers to the angular FWHM of the XRD peak at the diffraction angle θ [20]. The FWHM is 0.119 at the θ value of 38.03°, and the particle size is 84.8 nm. The similar crystalline for the silver nanoparticles by using - microwave-assisted green synthesis has been documented in the literature studies [21].

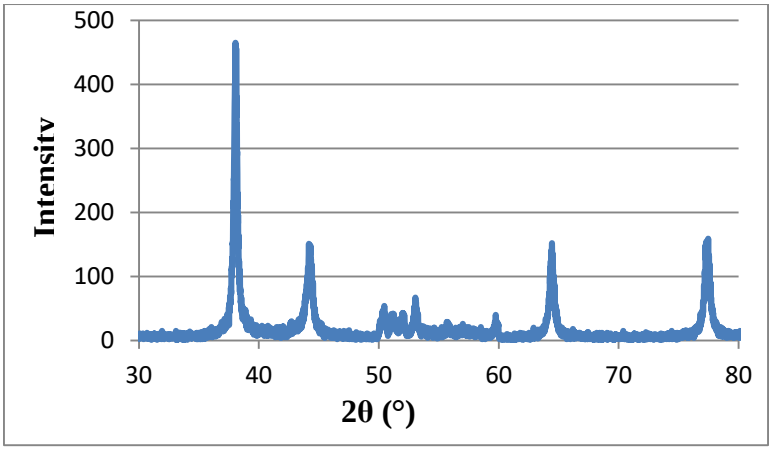
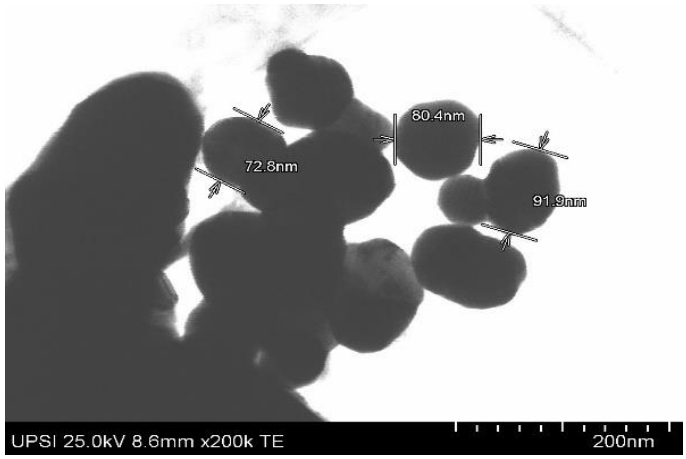


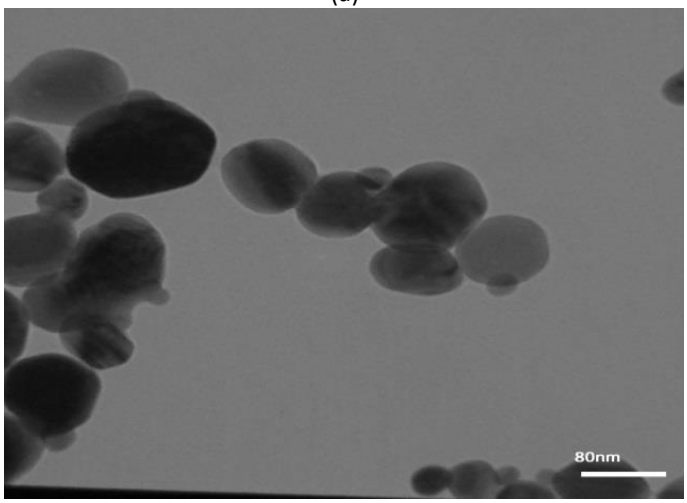
Fig. 5. XRD spectra of synthesized AgNPs

3.5 TEM (Scanning Transmission Electron Microscopy) and STEM (Transmission Electron Microscopy)

STEM was used to confirm the size and shape of AgNPs. The STEM image in Figure 6(a) shows a spherical shape for AgNPs, and the measured average size ranged from 72 nm to 100 nm. The TEM image in Figure 6(b) demonstrates the precise shape for the particles.



(a)



(b)

Fig. 6. (a) scanning transmission electron microscopy, and (b)transmission electron microscope

3.6 Antibacterial Activity for AgNPs

The AgNPs antibacterial properties were affirmed based on the method of disk diffusion, as mentioned previously. A ruler was used to measure the ability of the AgNPs to prevent bacterial growth against both bacteria. For the negative control, distilled water was used while for positive control, and the ampicillin was used and checked against bacteria. Figure 7 shows the inhibition zone of *E. coli* as Gram-negative bacteria and *S. aureus* as Gram-positive bacteria. The distilled water inhibition was zero in both bacteria. The inhibition zones of the extract were 12.2 and 10.4 mm in contrast to *E. coli* and *S. aureus*, respectively. The inhibition zones of ampicillin were 28.6 and 22.1 against *E. coli* and *S. aureus*, respectively. Different AgNPs concentrations resulted in functional inhibition zones against both bacteria. The inhibition zone increased with AgNPs concentration. Table 1 listed the detail of AgNPs inhibition and Table 2 listed the control samples.

Table 1
Antibacterial activities of AgNP

Bacteria	Ampicillin	Plant extract	Distilled water
E. coli	28.6 mm	12.2	0
S. aureus	22.1 mm	10.4	0

Table 2
Control sample against E. coli and S. aureus

AgNPs	*E. coli*	*S. aureus*
10 µg/ml	14.6 mm	11.1 mm
20 µg/ml	16.1 mm	13.6 mm
40 µg/ml	17.9 mm	16.5 mm
60 µg/ml	20.5 mm	17.6 mm
80 µg/ml	26.8 mm	19.2 mm

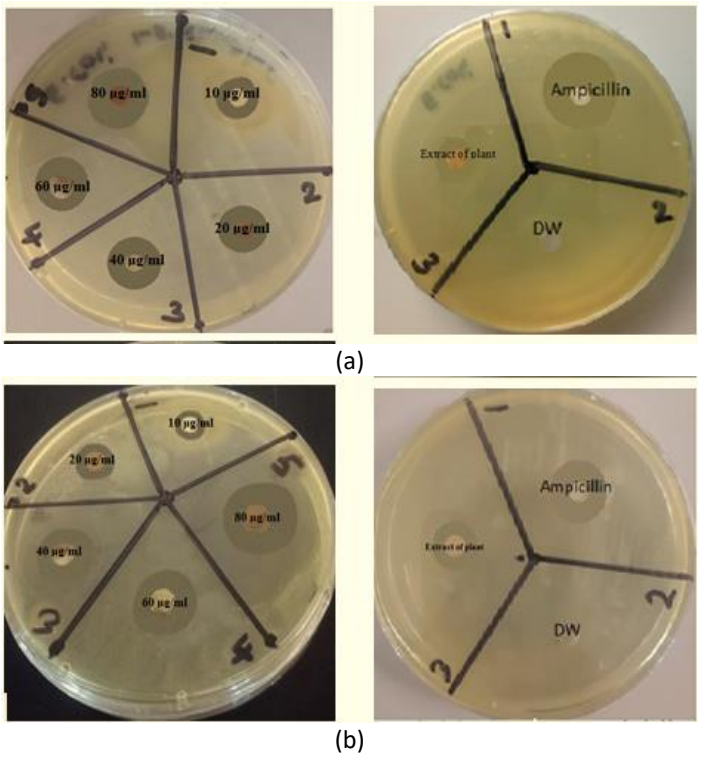


Fig. 7. Antibacterial activities of (a) AgNPs and control samples against *E. coli* and (b) AgNPs against *S. aureus*

4. Conclusions

Biosynthesis methods have attracted the attention of researchers due to their characteristics, such as safety, low cost, and environmental friendliness. However, these methods are considered slower techniques than chemical ones. Green microwave synthesis, a rapid technique due to the use of microwave, was employed in the present study to synthesize AgNPs from biological sources. *M. dubia* (neem) leaves were used as a natural source. AgNPs were successfully synthesized and categorized using UV–vis, SEM, XRD, EDX, STEM, and TEM. The morphology and particle size were also investigated. These techniques obtained a spherical shape with average sizes ranging from 70 nm to 100 nm. The antimicrobial characteristics of the synthesized AgNPs were investigated on the Gram-positive and negative bacteria. It was discovered that using AgNPs as an agent for microbes was highly efficient, and it has the potential to be used in the medical field as an antibacterial agent in the future.

Acknowledgement

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