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Physical Characterization of Plant-Mediated AgNPs-Ei Synthesized using *Eleusine indica* Extract and their Antibacterial Properties

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This study is on the biomimetic synthesis of plant-mediated silver nanoparticles (AgNPs-Ei) using *Eleusine indica* methanol extract. Phytochemical analysis was done qualitatively to determine the presence of active metabolites such as alkaloids, flavonoids, phenols, glycosides, quinone, steroids, saponin, tannin and terpenoid. Antibacterial activity of AgNPs-Ei against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Enterobacter aerogenes* were analysed using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays. *E. indica* methanol extract was treated with 1 mM of silver nitrate at room temperature (25-27°C) for 24 hours. The phytochemical analysis of *E. indica* methanol extract shows several phytochemicals, including alkaloid, flavonoid, phenols, saponin, tannin and terpenoid. Further, the biomimetically synthesized plant-mediated silver nanoparticles (AgNPs-Ei) morphology was characterized using UV-Vis spectroscopy, TEM and FESEM-EDX. UV-Vis absorption spectroscopy shows a strong resonance centred on the surface of AgNPs-Ei at approximately 413 nm. The physical appearance of AgNPs-Ei was characterized by TEM analysis with an average particle size of 20 nm. FESEM-EDX determined the morphology of elemental silver in the spherical shape and the peak of Ag at 3 keV. Apart from that, the antibacterial activity of AgNPs-Ei has predicted bacteriocidal activity according to the ratio of MBC to MIC values against tested Gram-positive and Gram-negative bacteria. This study revealed that the AgNPs-Ei has been successfully synthesized by reduction of silver nitrate with *E. indica* methanol extract and showed a potential antibacterial agent.

Keywords: AgNPs-Ei; *Eleusine indica*; UV-Vis analysis; FESEM-EDX; antibacterial activity

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Silver nanoparticles (AgNPs) are considered of great importance due to their high antivirus, antibacterial, anticancer, and antifungal properties [1] and show good potential in biomedical applications [2]. Conventional methods for synthesizing AgNPs, such as physical and chemical procedures, involve using heavy equipment, substantial energy consumption, and highly toxic and hazardous chemical compounds that can cause biological hazards [3]. Alternatively, biomimetic nanoparticle syntheses using natural resources such as plants, fruit, fungi, and algae are suitable because they are simple, cost-effective, and eco-friendly [4]. Also, biomimetic nanoparticles are valuable for a variety of fields, including optoelectronic devices [5], catalytic activities [6], the food packaging industry [7] and be regarded as the agent of wound-healing, antioxidant, anti-microbial, antiviral, and anticancer [2].

The plant extract was commonly used in bio-mimetically nanoparticle synthesis because it contains a wide range of metabolites that can act as capping, reducing, and stabilising agents [3]. Several plants have been used in synthesizing AgNPs, including *Ramalina dumeticola* [8], *Prunus persica* [9], *Ficus hispida Linn* [10], *Callistemon citrinus* [2], *Crocus Haussknechtii Bois* [6] and *Canarium patentinervium* [11]. In this study, *the Eleusine indica* or Sambau plant is studied as a plant candidate for the synthesis of AgNPs-Ei. The strategy aims to produce plant-mediated silver nanoparticles (AgNPs-Ei) by using *E. indica* methanol extract as a reductant and stabiliser. Further, the physicochemical and biological properties of AgNPs-Ei were investigated.

EXPERIMENTAL

Chemicals and Materials

The fresh leaves of *Eleusine indica* were collected in Bangi, Selangor and Kuala Pilah, Negeri Sembilan. This species was authenticated by Forest Research Institute Malaysia (FRIM), where a voucher specimen (FRI-51486) was deposited. All the reagents purchased were of laboratory grade and used as received. Silver

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nitrate ($AgNO_3$) was purchased from R&M Chemicals, US. Methanol was purchased from Sigma Aldrich, Germany.

Extraction and Phytochemical Analysis of *Eleusine indica*

About 250 g of *Eleusine indica* leaves powder was soaked in 2500 mL of methanol for 72 hours at room temperature for the cold extraction method [12]. The crude extracts of *E. indica* were obtained using a vacuum rotary evaporator and kept in a vial for further analysis. The present of metabolites in the plant extract was screened through several phytochemicals test [13,14].

Biomimetically Synthesis of AgNPs-Ei

Biomimetically synthesis of plant mediated silver nanoparticles using *E. indica* (AgNPs-Ei) was followed method by [15] with some modification. About 0.1 g *E. indica* was dissolved in 10 mL methanol extract and was added to 30 mL of 1 mM silver nitrate solution. The mixture was allowed to react for 24 hours at room temperature (24-25ºC). The appearance of yellowish-brown color of the solution indicates the formation of AgNPs [8].

Characterization of AgNPs-Ei

Physicochemical properties of AgNPs-Ei were characterized using ultra-violet visible spectra (UV-Vis), transmission electron microscopy (TEM) and field emission scanning microscopy electron – energy dispersive X-ray analysis (FESEM-EDX). The UV-Vis spectra analysis was conducted using a UV 2450 Shimadzu double-beam spectrophotometer, operated at 2 nm from 300 to 500 nm. The particle size of AgNPs-Ei was confirmed by TEM. TEM analysis of AgNPs-Ei was performed using a Philips CM12 instrument operated at an accelerating voltage at 80 kV. The size distribution of the AgNPs-Ei was calculated from the TEM images by measuring the diameter in nm of approximately 50 nanoparticles. The morphology of elemental silver in the AgNPs-Ei was determined by FESEM-EDX, through the ZEISS model Merlin that operated at an accelerating voltage of 10 kV.

Evaluation of Antibacterial Activity of AgNPs-Ei

The antibacterial potential of the AgNPs-Ei was determined using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays [16]. In the assay, two Gram-positive bacteria (*S. aureus*, *B. subtilis*) and two Gram-negative bacteria (*E. coli*, *E. aerogenes*) were used against AgNPs-Ei. MIC assay was performed triplicate in a 96-well microtiter plate with serial double dilutions of plant-mediated silver nanoparticles (AgNPs-Ei) varying from 100% to 1.562 % in nutrient broth and was incubated at 37ºC for 24 hours. After 24 hours, 10 µL of 0.2 mg/mL 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution was added to each well and incubated for another 2 hours. The solution colour change from pale yellow to red/purple indicated biologically active bacteria [16]. MBC is the determination of the lowest concentration without colony growth on the agar plates. It was determined by plating 10 µL from each well that showed no growth on a Mueller Hinton agar plate. Plates were then incubated for a further 24 hours at 37°C.

RESULTS AND DISCUSSION

The phytochemical analysis, physicochemical properties and antibacterial activity of biomimetically AgNPs-Ei were successfully done, and it is reported in this section.

Extraction and Phytochemical Analysis of *E. indica*

Dried ground *E. indica* leaves was used in this study to enhance the surface area and optimize the extraction process and we found that the percentage yield of the crude extract was calculated as 5.32 %. Further, analyzing bioactive compounds from plants is essential to ascertain their medicinal value [17]. This study found that pharmacologically active compounds such as alkaloids, flavonoids, phenolics, saponin, tannin and terpenoids were present in the sample (Table 1).

Table 1. Phytochemical screening *E. indica* crude extract.

Phytochemicals test	Result
Alkaloids	+
Anthraquinones	-
Flavonoid	+
glycosides	-
Phenols	+
Quinones	-
Saponin	+
Steroid	-
Tannin	+
Terpenoid	+

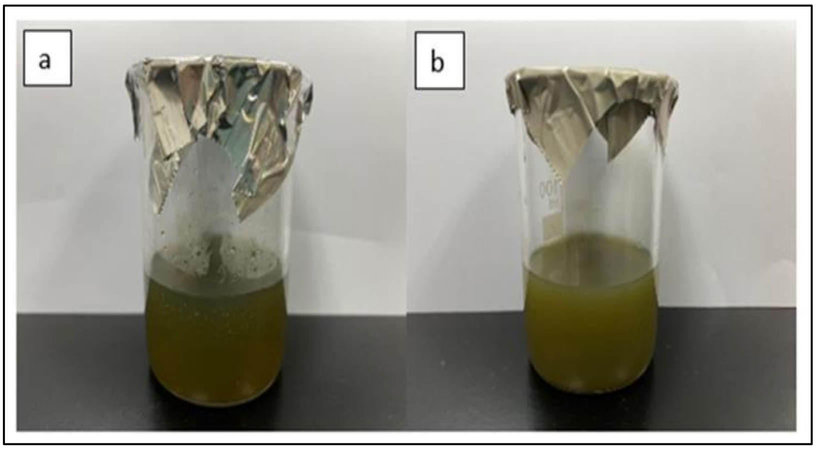

Figure 1. *E. indica* extract (a), AgNPs-Ei 24 hours (b).

The finding of phytochemicals of *E. indica* methanol extract is similar to the previous study reported by [18]. It has been reported that *E. indica* extract contains 12 metabolites such as hydroxycinnamic acid, naringenin (flavanones), 2(3,4-dihydroxyphenyl)-7-hydroxy-5-benzenepropanoic acid, antraquinone, caffeic acid derivative, caffeoyl glucose, hydroxy-benzoic acid derivatives methyl 2-[cyclohex-2-en-1-yl(hydroxy)methyl]-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxoprolinate (aglycone) from the flavonoid and phenolic groups [19]. This study suggested that flavonoid and phenolic metabolites of *E. indica* as a potential reducing and stabilizing agent in the synthesis of AgNPs-Ei. According to [20] suggest that some metabolites such as flavonoids, phenolic compounds, sugar, and proteins can serve as reducing and stabilizing agent in synthesizing AgNPs.

Biomimetically Synthesis of AgNPs-Ei

The formation of AgNPs-Ei was shown by a yellowish colour solution from the mixture of *E. indica* methanol extract and 1 mM $AgNO_3$ solution, which was caused by the excitation of surface plasmon resonance in the AgNPs [9]. Figure 1 shows the colour changes when the *E. indica* methanol extract was mixed with a 1 mM $AgNO_3$ solution and treated for 24 hours at room temperature. This reduction of silver ions to silver nanoparticles could be attributed to -OH groups in flavonoids like naringenin, which can be produced during the tautomeric shift of flavonoids from the enol to keto-type, releasing reactive hydrogen atoms that cause silver nanoparticle reduction [10]. The exact mechanism of the reduction of silver ion (Ag^+) is still under investigation.

Characterization of AgNPs-Ei

The physicochemical characterization of synthesized AgNPs-Ei was done using UV-Vis spectra, TEM and FESEM-EDX analysis. AgNPs-Ei shows an optimum UV–visible absorption in the 400–500 nm range due to its surface plasmon resonance [21]. The surface plasmon resonance of the synthesized AgNPs-Ei was centred at 413 nm, indicating the presence of AgNPs in the solution (Figure 2). According to [22], AgNPs' absorbance is mainly determined by their size and shape.

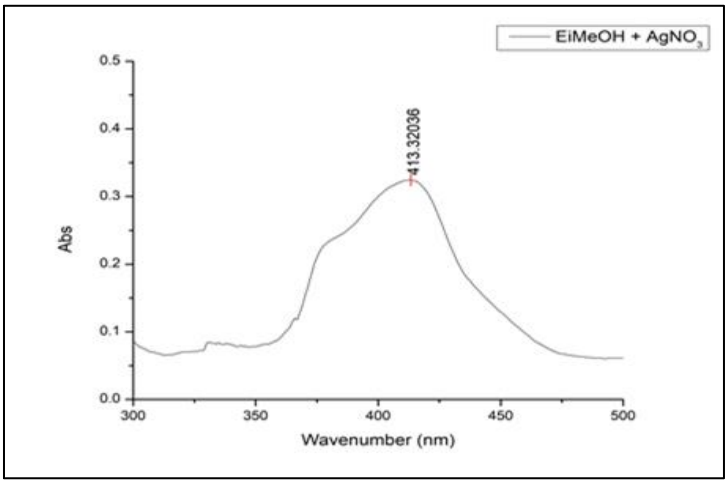

Figure 2. UV-Vis absorption spectrum of AgNPs-Ei.

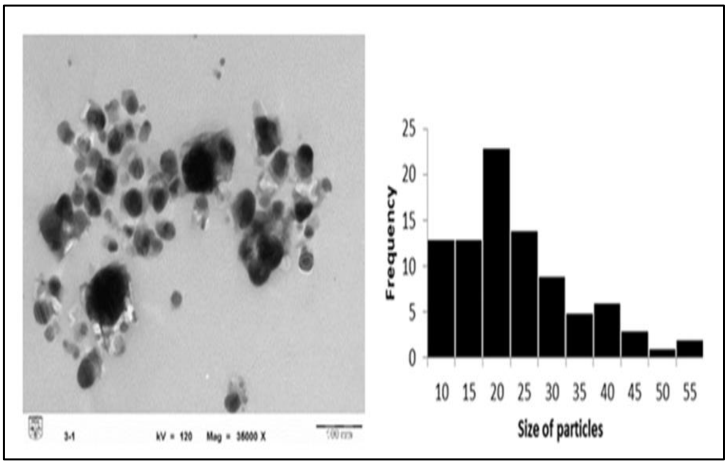

Figure 3. TEM micrograph and particle size histogram of AgNPs-Ei.

TEM micrograph and the particle size histograms of AgNPs-Ei show that the particles are almost spherical with an average size of 20 nm (Figure 3). Previously, silver nanoparticles synthesized from *Vigna radiata L.* had an average size of 50 nm [23]. In addition, [24] was found that AgNPs synthesized using *Ziziphora tenuior* extract has an average size of 8 to 40 nm. This shows that AgNPs, have varying scale sizes depending on the type of metabolites in the plant extracts [8]. The defined size and morphologies of AgNPs is very challenging due to the high number of reaction parameters that must be monitored and controlled [25].

The biomimetically synthesized plant-mediated silver nanoparticles (AgNPs-Ei) morphology was further characterized using FESEM-EDX. The micrograph FESEM with the magnification of 10000x is shown in Figure 4. It reveals that the formation of AgNPs-Ei is less than 100 nm with a spherical shape. Energy dispersive X-ray spectroscopy (EDX) is used to gain further insight into the features of AgNPs-Ei. In the EDX spectrum of AgNPs-Ei, an optical absorption characteristic peak of Ag is present at 3 keV corresponding to the binding energies of AgL [26]. Some impurity peaks were detected below 1 keV, corresponding to a carbon peak (CK) and oxygen peak (OK), which was probably related to crystalline biomolecules in the *E. indica* extract.

Evaluation of Antibacterial Activity of AgNPs-Ei

In the field of nanotechnology, applications of AgNPs as antibacterial have been expanding. Therefore, the antibacterial activity of the synthesized AgNPs-Ei against two Gram-positive (*S. aureus*, *B. subtilis)* and two Gram-negative (*E. coli*, *E. aerogenes)* bacteria were investigated through Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays (Table 2). For a comparison, we found that MIC and MBC assay of *E. indica* extract against tested bacteria occur at higher concentration of 25.00 ug/mL. All tests were done in triplicates.

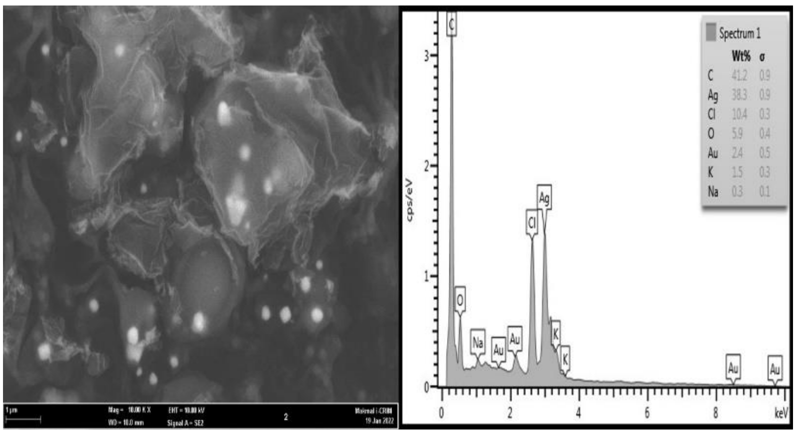

Figure 4. FESEM micrograph and EDX analysis of AgNPs-Ei.

Table 2. MIC and MBC assay of AgNPs-Ei against tested bacteria.

Bacteria	MIC (µg/mL)	MBC(µg/mL)	MBC to MIC ratio
Staphylococcus aureus	12.50 (± 0.02)	12.50 (± 0.01)	1
Bacillus subtilis	1.56 (± 0.01)	1.56 (± 0.01)	1
Escherichia coli	6.25 (± 0.02)	12.50 (± 0.02)	2
Enterobacter aerogenes	3.13 (± 0.02)	6.25 (± 0.01)	2

As a result, AgNPs-Ei has predicted bactericidal activity due to the ratio of MBC to MIC, which is less than 4. According to [11], the antibacterial effect was considered bactericidal if the ratio MBC/MIC was less than 4, and the effect was called bacteriostatic if the ratio MBC/MIC was more than 4. The antibacterial effects of AgNPs could be determined by several factors, such as the characteristics of certain bacterial species, the type of metabolite present in the plant [27] and the size and shape of AgNPs [28]. The exact mechanism by which silver ions and AgNPs exert their antibacterial effect remains to be identified.

CONCLUSION

In conclusion, the AgNPs-Ei has been successfully synthesized through a biomimetic process by reduction of silver nitrate with *E. indica* methanol extract. The phytochemical analysis of *E. indica* methanol extract indicates the presence of alkaloid, flavonoid, phenols, saponin, tannin and terpenoid. The AgNPs-Ei morphology shows a strong UV-Vis resonance centred at approximately 413 nm with an average particle size of 20 nm. FESEM-EDX determined the morphology of elemental silver in the spherical shape and the peak of Ag at 3 keV. AgNPs-Ei also shows a potential antibacterial agent against *S.aureus*, *B. subtilis*, *E. coli*, and *E. aerogenes*. These studies may lead the way for a new range of antibacterial agents and the possibility of using AgNPs as antiviral agents.

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