



Synthesis of Silver Nanoparticles using *Acalypha indica* Leaves Extracts and Its Antioxidant Activity

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Abstract

The leaves were extracted using the maceration method and acted as both reducing agent in the nanoparticle synthesis. The formation of AgNPs was indicated by a color change from green to reddish-brown and confirmed by Ultraviolet-Visible spectroscopy with a characteristic absorption peak around 420 nm, which corresponds to surface plasmon resonance (SPR). Antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl, DPPH radical scavenging assay. The antioxidant activity increased in dependence on concentration with the 10 mM AgNP solution exhibiting the greatest inhibition at 84.27%. With an estimated IC₅₀ value of 255.3 ± 3 µg/mL, the substance was shown to have moderate antioxidant activity. The synthesis of nanoparticles and antioxidant potential were both enhanced by the presence of active phytochemicals such as flavonoids, terpenoids, and tannins found in *A. indica*. As a sustainable substitute for traditional chemical processes, our study demonstrates that *A. indica* is a reliable and environmentally friendly source for the biosynthesis of AgNPs. The produced AgNPs have antioxidant qualities, indicating possible uses in the pharmaceutical and biomedical industries.

Keywords: nanoparticles; 2,2-Diphenyl-1-picrylhydrazyl; *Acalypha indica*

Introduction

Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nm, where unique phenomena enable novel applications. It involves the design, development and application of materials with the smallest functional components [1]. As a general-purpose technology, nanotechnology has wide-ranging applications in the medical, pharmaceutical, food, agriculture and environmental sectors. Nanochemistry combines chemistry and nanoscience to produce nanoscale materials of various sizes, shapes and functions. Nanoparticles, sized between 1 to 100 nm, possess small size, high surface area, catalytic activity, chemical stability and multiple reactive sites [2]. Among them, silver nanoparticles (AgNPs) are popular and can be synthesized using electrochemical, thermal, hydrothermal, and green methods. Compared to bulk materials, nanoparticles offer a higher surface area and altered mechanical, electrical, and chemical properties due to quantum size effects [3].

Green synthesis is a preferable alternative to chemical and physical methods, as it is more cost-effective, environmentally friendly, and avoids toxic chemicals, high temperature, and pressure. AgNPs produced via green synthesis have shown antibacterial properties and, when combined with antibiotics, are even more effective against bacterial infections [4]. Plants such as *Acalypha indica* are rich in bioactive compounds like flavonoids, tannins, phenols, and alkaloids, which can act as reducing and stabilizing agents in nanoparticle synthesis [5]. Several studies have used *A. indica* extracts to successfully synthesize AgNPs and evaluate their antioxidant and antimicrobial activities [6]. The presence of secondary metabolites contributes to enhanced biological properties, making it an ideal candidate for nanoparticle synthesis. In this study, silver nanoparticles were synthesized using *A. indica* leaf extract via a green approach. The synthesized AgNPs were characterized using Ultraviolet-Visible spectroscopy and assessed for their antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl, DPPH assay. This method also explores the potential biomedical applications of *A. indica* derived nanoparticles.

Methodology

General Experimental Procedures

The cold maceration method was used to extract the leaves of *Acalypha Indica*. (UV-VIS) were then used to characterise the silver nanoparticles. After that, the DPPH radical scavenging assay was used to assess antioxidant activity.

Plant Material

The leaves of *A.indica* were collected from Selangor. The leaves were continuously cleaned in double-distilled water after being twice washed with tap water to remove dust particles from their surface. After washing, the leaves were left in the shade for five days to make it dried and need to avoid direct sunlight. [6]. Before getting used for the extraction procedure, the leaves were then cut into pieces and crushed into a powder using a grinder machine.

Extraction of A. indica leaves

The dried leaves (5g) was weighed and were crushed with 100 mL of methanol were added [7]. The mixture was then incubating in dark place with room temperature for 3 days. The mixture was filtered and the reddish brown color. Whatman filter paper was used to filter the mixture. Thus, (AgNPs) were made and the extract solution was deposited at 4°C and used to synthesise AgNPs.

Synthesis of Silver Nanoparticles (AgNPs) by using A.indica leaves extract

In the first step, for this experiment, the synthesis of the silver nanoparticles was modified from [8]. For the 0.01M concentration, 0.017 g of liquid silver nitrate (AgNO_3) was mixed with 10 ml of distilled water using Erlenmeyer flask. After that, the solution was diluted into 5 different concentrations ranging between 2 mM to 10 mM. 5mL of silver nitrate solution (AgNO_3) were mixed with 2 mL of *A.indica* extract solution. The solution was stirred continuously for 24 hours at 35°C, at which point the reaction began and the solution changed colour from green to intense reddish brown that shows the presence of silver nanoparticles in the solution. Using a UV-vis spectrophotometer, the mixture was analysed at wavelengths between 300 and 700 nm throughout several incubation times and the absorption peak was noted [9].

Characterization of Silver Nanoparticles (AgNPs)

For characterization, the biosynthesis of AgNPs sample was characterized by measure and observe the UV absorbance at 300-700 nm wavelengths using UV-visible spectrophotometer. The production of silver nanoparticles was confirmed by the observation of the absorption peak [6].

Ultraviolet Visible Spectroscopy (UV-Vis)

UV-vis spectroscopy is used to determine the absorbance spectra of a substance in solution or as a solid. Spectroscopy observes the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the compound's or material's first singlet excited state [3]. UV-Vis spectrophotometers detect the absorption or transmission of light as it passes through a medium based on wavelength. UV-Vis spectrophotometer techniques are useful in a variety of research disciplines, including agriculture, food, pharmaceuticals, the environment and others. 5 different concentrations which are 2,4,6,8 and 10 mM were used to estimate absorbance values ranging from 300 to 700 nm.

DPPH Free Radical Scavenging Assay

In this experiment, the antioxidant activity of silver nanoparticles (AgNPs) synthesized using *A. indica* leaf extract was evaluated using the DPPH radical scavenging assay. The AgNPs used were not purified into powder form and were used directly in solution which contains both silver nanoparticles and residual plant extract. 0.1 mM DPPH solution was prepared by dissolving 0.001 g of DPPH in 30 mL of methanol. Five different concentrations of AgNP-extract solution (2, 4, 6, 8, and 10 mM) were prepared. For each sample, 1 mL of AgNP solution was mixed with 2 mL of DPPH solution and incubated in the dark for 30 minutes at room temperature. A blank was prepared by mixing 2 mL of DPPH solution with 1 mL of methanol. After incubation, the absorbance of each sample and the blank was measured at 517 nm using a UV-Vis spectrophotometer with a quartz cuvette [10].

Results and Discussion

Extraction of A. indica leaves

The dried leaves (5 g) were weighed and crushed using grinder machine. 100 mL of methanol were added into the dried leaves that have been crushed. The maceration method was used for the extraction. The mixture was then incubating in dark place with room temperature for 3 days. The mixture was filtered and the reddish brown color of leaves extract was stored under 4°C in the fridge and used to synthesize silver nanoparticles.

Synthesis of Silver Nanoparticles using A. indica extracts

In this study, an extract from *A. indica* leaves was used to synthesis silver nanoparticles. Five different concentrations of the *A. indica* extraction which are 2,4,6,8 and 10 mM of AgNO₃ were used. When *A. indica* leaves extract (2 mL) was added to the aqueous silver nitrate solution, AgNO₃ (5 mL), it turned reddish brown in more than 24 hours. The longer the incubation period, the more intense the brown coloration. Over the course of the incubation time, the reddish brown colour increased because of the reduction of AgNO₃ and the stimulation of surface plasmon resonance (SPR) [11]. On the other hand, the leaf extract-free control AgNO₃ solution did not exhibit any colour change. Figure 1 shows the solutions colour changed from green to reddish brown within 24 hours of incubation at room temperature. The synthesis of AgNPs were kept in a dark container to maintain their stability. This indicated the formation of silver nanoparticles due to the reduction of silver ions. The synthesis of silver nanoparticles was further verified using UV-Vis characterization methods.

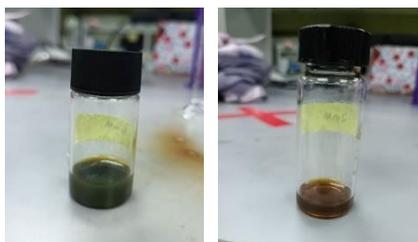


Figure 1 Solutions colour changed from green to reddish brown within 24 hours

Ultraviolet Visible Spectroscopy (UV-Vis) for A. indica leaves extracts

Five different concentrations which are 2, 4, 6, 8 and 10 mM were used to estimate absorbance values ranging from 300 to 700 nm. In previous study, the production of silver nanoparticles was confirmed by the UV-Vis spectrums specific absorption peak at 420 nm [12]. UV-visible spectroscopy is a common method for analyzing nanoparticle production because of its sensitivity to the surface plasmon resonance (SPR) phenomenon. Silver nanoparticle formation was shown by a distinct color change from green to reddish-brown within 24 hours of the *A. indica* leaf extract with different concentrations of AgNO₃ (2 – 10 mM). The UV-Vis spectra of the formation of AgNPs showed absorption peaks about 420 nm which is consistent with previous study on silver nanoparticle synthesis with plant extracts. This absorption peak is due to SPR of silver nanoparticles which is a group resonance of electrons that conduct produced by light at specified wavelengths. The SPR band indicates the reduction of Ag⁺ ions to elemental Ag⁰ and the formation of nanoparticles.

The intensity of the spectra changed with the quantity of AgNO₃ shows a correlation with nanoparticle yields. Higher concentrations which were 8 and 10 Mm produced stronger SPR bands makes it increased nanoparticle formation. However, it is important to note that too high concentrations may cause aggregation or formation of polydispersed particles thereby broadening or shifting the SPR peak. The narrowness and concentration of the SPR peak at 420 nm indicate that the formation nanoparticles were generally monodisperse and spherical in shape as SPR is extremely sensitive to particle size, shape and distribution (Figure 2). To summarize, UV-Vis spectroscopy showed the successful green synthesis of silver nanoparticles using *A. indica* leaf extract. The presence of a significant SPR band near 420 nm at all tested concentrations confirms the synthesis of AgNPs and demonstrates the plant extracts efficiency as a natural reducing and stabilizing agent.

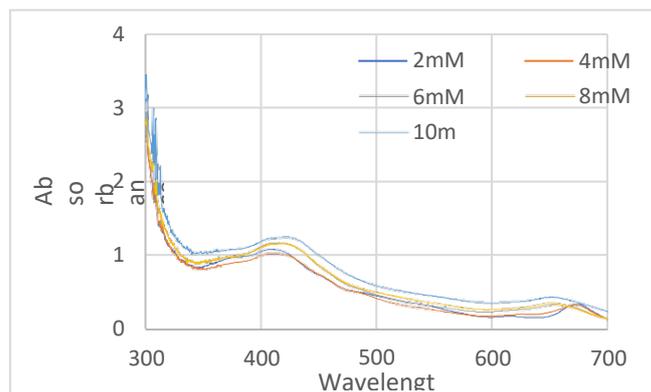


Figure 2 UV-Vis spectroscopy in different concentration

Antioxidant Activity of Biological Silver Nanoparticles

The antioxidant activity of silver nanoparticles (AgNPs) produced *A. indica* was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test. The synthesized AgNP solution was not dried to powder in this research due to difficulties in the separation and drying stages, especially with the presence of plant extract residues. As a result, the colloidal AgNP solution containing both nanoparticles and phytochemicals from the extract was examined directly for antioxidant activity. Various concentrations of AgNPs (2 mM to 10 mM) were prepared and reacted with 0.1 mM DPPH in methanol. Each concentration was mixed with the DPPH solution and incubated in the dark for 30 minutes to prevent light-induced degradation of the DPPH radical. The absorbance of the mixtures was recorded at 517 nm using a UV-Vis spectrophotometer. The blank consists of DPPH solution in methanol without any AgNP sample.

The results showed a gradual increase in DPPH radical inhibition with increasing AgNP concentration. The inhibition percentages ranged from 47.22% at 2 mM (215.74 $\mu\text{g/mL}$) to 84.27% at 10 mM (1078.7 $\mu\text{g/mL}$), indicating a concentration-dependent antioxidant effect (Figure 3). This confirms the capability of AgNPs synthesized using *A. indica* to scavenge free radicals effectively. The IC_{50} value was approximately $\pm 255.30 \mu\text{g/mL}$, which is considered moderate antioxidant activity. The antioxidant activity can be attributed to both the metallic silver nanoparticles and the bioactive chemicals found in the *A. indica* extract that remained in the solution. The presence of these phytochemicals most likely increased the radical scavenging effect. Although the AgNPs were not developed into a solid powder, the colloidal suspension still shows significant antioxidant activity, to support previous results [10]. In conclusion, the synthesized AgNPs shown good DPPH radical scavenging, even in the presence of plant extracts. Future optimization, such as purification or drying, may enhance reproducibility and allow for more accurate comparisons with typical antioxidant compounds.

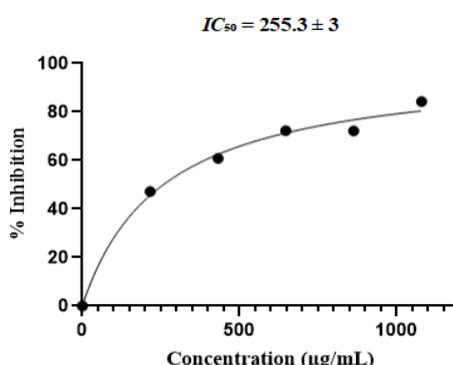


Figure 3 Percentage of Radical Scavenging Activity against concentration of synthesized *A. indica*-AgNPs leaves

Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using

Acalypha indica leaf extracts and evaluated their antioxidant activity. The maceration method provided an efficient extraction process for the bioactive phytochemicals such as flavonoids, terpenoids, alkaloids and tannins, which act as natural reducing and stabilizing agents in the biosynthesis of AgNPs. The synthesized nanoparticles were characterized using UV-Vis spectroscopy, with absorption peaks observed around 420 nm, confirming the formation of AgNPs and indicating their spherical and monodisperse nature. The change in solution color from green to reddish-brown further supported the successful reduction of silver ions.

The DPPH radical scavenging experiment was used to assess the synthesis of AgNPs antioxidant properties. With an IC₅₀ value of approximately 255.3 ± 3 µg/mL, the results showed a concentration-dependent increase in radical scavenging activity. This moderate antioxidant activity implies that the AgNPs effectiveness was increased by the retention of bioactive components from the *A. indica* extract. Overall, this study demonstrates *Acalypha indica*'s potential as a cost-effective, environmentally friendly, and sustainable solution for silver nanoparticles with antioxidant properties. In addition to reducing the usage of hazardous chemicals, the green synthesis method supports sustainable development and green chemistry.

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