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Research Paper

Effect of Different Process Parameters on the formation of Silver Nanoparticles using Crude and Modified Neem (*Azadirachta indica)* Leaf Extracts

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Abstract

In recent times, the biosynthesis of nanoparticles, which has led to significant growth in the field of nanotechnology. The use of plant extracts has become an impetus in this field as it is a simple and eco-friendly method. This study was an attempt to study different parameters in biosynthesis of silver nanoparticles using *Azadirachta indica* (Neem) leaf extracts. Four different process parameters such as concentrations of neem leaf extract, types of neem leaf extract, mixing ratios and the reaction time period were investigated on the formation of silver nanoparticles. Initially, the formation of silver nanoparticles was detected by the visual observation. Then, the synthesized silver nanoparticles were characterized using UV-Visible spectroscopy and scanning electron microscopy (SEM). The change of color from yellow to reddish brown color confirmed the formation of silver nanoparticles. The silver surface plasmon resonance (SPR) band obtained in the expected visible range of UV-Visible spectroscopy confirmed the synthesis of the nanoparticles. SEM images showed that silver nanoparticles are roughly spherical and of uniform particle size, and the average particle size is 100 nm. Further, the maximum absorbance of SPR band was considerably varied with different process parameters used in the present study. The UV-Visible spectra of 2.5 g/100 mL of crude neem leaf extract without any dilution showed maximum absorbance in the expected range with the mixing ratio of (Neem and $AgNO_3$) 1:8. However, the maximum absorbance of modified neem leaf extracts (pH 10) resulted lower in value than the crude extracts in the 20 times diluted sample with the mixing ratio of 1:9. Moreover, modified extract with UV radiation exposure increased the absorbance in the expected visible range. It concludes that fine tuning of the bioprocess parameters would enhance nanoparticle synthesis.

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Keywords: Modified neem leaf extract, silver nanoparticles, surface plasmon resonance, UV-Visible spectroscopy

Introduction

Nanotechnology is one of the most prominent areas in modern research. The synthesis of nanoparticles has drawn a considerable attention in recent times with regard to their tremendous applications in various fields such as diagnostics [1], antimicrobials and therapeutics [2-3]. Among all nanoparticles, metallic silver nanoparticles are most promising as they show excellent antibacterial properties, which is emerging as the current interest in many researchers due to the increasing public health problem.

A number of methods are available for the synthesis of silver nanoparticles such as chemical and photochemical reactions in reverse micelles [4-5], thermal decomposition of silver compounds [6], chemical reduction with or without stabilizing agents [7]. Recently green synthesis of nanoparticles [8-10] is a quite novel approach leading to truly eco-friendliness, which provides greater advancements over other approaches, including chemical and physical methods.

Therefore, biosynthesis of synthesizing nanoparticles is considered to be an environmentally benign technology that does not use high pressure, energy, temperature and toxic chemicals in the synthesis protocols. In recent times, researchers have achieved success in the synthesis of silver nanoparticles using several plant extracts such as *Camellia sinensis* [11], *Magnolia kobus* and *Diopyros kaki* leaf [5], *Acalypha indica* leaf [12] and *Gliricidia sepium* [13]. *Azadirachta indica* leaf extract has also been used for the synthesis of silver, gold and bimetallic (silver and gold) nanoparticles as it is a commonly available medicinal plant and the biosynthesized silver nanoparticles have efficient antibacterial activity as it was capped with the neem leaf extract [14],[10].

The present study focused on investigating the effect of process variables like reductant concentration, type of neem extracts (crude and modified), mixing ratio of the reactants and reaction time on silver nanoparticles formation using different neem leaf preparation.

Methodology

Different Concentrations of Neem Leaf Extracts

Preparation of Crude Neem Leaf Extract

Different concentrations of neem leaf broth were prepared by taking different amounts (1, 2.5, 5 and 10 g) of disease free, fresh leaves of neem (*Azadirachta indica*).

The fresh leaves were washed thrice with sterile distilled water. Then the leaves were finely cut and boiled for 2 min with 100 ml of sterile distilled water in a 500 ml Erlenmeyer flask.

Preparation of Modified Neem Leaf Extract

In another variation of the synthetic process, the modified neem leaf extract was used as it has been previously confirmed for antibacterial activity [15]. The modified extract was prepared by adjusting the pH of the crude neem leaf extract of 2.5 g/100 ml into different pH and the extract was filtered to remove the contaminants from the crude extract. As the modified neem leaf extract prepared at pH 10 was found to be the most effective for the antibacterial activity, it was used for the preparation of silver nanoparticles in the present study.

Mixing Ratios of Neem Leaf Extracts

The silver reduction reaction was carried out at different mixing ratios of both crude and modified leaf extracts of neem (after 20 times dilution) (5 ml) to different volumes (5 ml, 10 ml, 15 ml and 45 ml) of 0.001 M of silver nitrate solution without varying the other conditions. Further, the 2.5 g/100 ml of crude neem leaf extract (without any dilution) was also carried out at the mixing ratio of 1:8 to get a better resolution.

Reaction Time of Neem Leaf Extracts

To find out the better reaction time needed for the biosynthesis of nanoparticles, the crude neem leaf extract (2.5 % concentration) was interacted with 0.001 M $AgNO_3$ in 1:8 mixing ratio for different time intervals ranging from 30 min to 120 min.

However, the modified neem leaf extract interacted with 0.001 M $AgNO_3$ in 1:9 mixing ratio was tested for three different time intervals 15 min, 30 min and 45 min after processing for UV exposure since the visual observation of the modified neem leaf extract showed different appearance during the incubation.

Characterization by UV-Visible Spectroscopy

The formation of silver nanoparticles was observed by the colour change and monitored by UV-Visible spectroscopy. UV-Visible spectroscopy analysis was carried out on Shimadzu double beam UV-Visible absorption spectrophotometer with a resolution of 1 nm between 200 to 800 nm [16].

UV Irradiation of Modified Neem Leaf Extract

To improve the efficiency of modified neem leaf extract, neem broth (2.5 % concentration) was mixed with 0.001 M $AgNO_3$ in different ratio as mentioned above and allowed to react for 48 hr until the colour change occured. Based on the colour change, the mixing ratio of 9:1 was processed further. Then, the mixture was exposed to UV light for 15 min, 30 min and 45 min respectively and characterized by UV-Visible spectrophotometer.

Characterization by Scanning Electron Microscopy (SEM)

Silver nanoparticles synthesized using the crude neem leaf extract were processed to determine the size of the nanoparticles. Silver nanoparticles were allowed to dry completely and ground well. Fixation was performed by incubation in a solution of a buffered chemical fixative gluteraldehyde. The dry specimen was mounted on a specimen stub using an adhesive epoxy resin or electrically conductive double-sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

Results and Discussion

Visual Observation and UV-Visible Spectroscopy

On adding different concentrations of the crude extract of neem (*Azadirachta indica*) to varying volumes of silver nitrate solution, the yellow color completely changed to reddish brown after 2 hr (Figure 1). The appearance of reddish brown color is due to the excitation of surface plasmon vibrations of typical of silver nanoparticles [17]; [16].

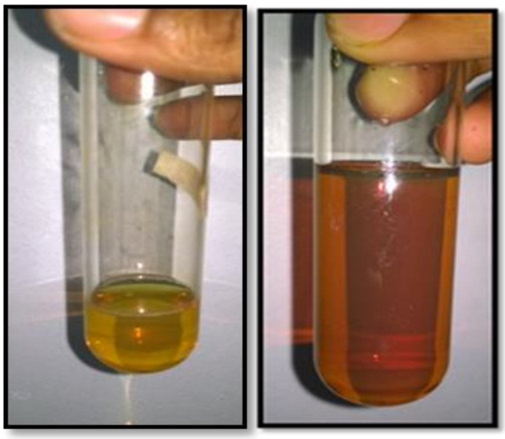

Figure 1. Solution of crude neem leaf extract before (right) and after exposure to the reaction with 0.001 M $AgNO_3$ (left)

In the present study, the formation of silver nanoparticles was further confirmed by UV–Visible spectroscopy and resulted the evidence of absorption in the UV -Visible range of 400–500 nm for the crude neem extract (Figure 2). Silver nanoparticles synthesized by neem leaf extract by different studies also resulted in λ_{max} values in the UV- Visible range of 400–500 nm [18].

In the present study, the UV -Visible spectra were recorded with varying amounts of plant extract (Figure 2). The nanoparticles synthesized in the concentration of 1 g/100 mL didn't result in a distinct absorption even though the surface plasmon resonance (SPR) of silver nanoparticles established in the expected range between 400 nm to 500 nm. However, the nanoparticles synthesized in the concentration increased to 2.5 g/100 mL resulted a distinct absorption of 0.326 after 2 hr of mixing reactants which corresponds to surface plasmon resonance (SPR) of silver nanoparticles established at 426 nm as shown in the Figure 2. On further increasing, the crude neem leaf extract concentration upto 5 g/100 ml and then 10 g/100 ml, there were no distinct absorption observed. On increasing concentration of extract, there is increase in intensity of absorption only upto 2.5 g/100ml. The variations in the values of absorbance signifies that the changes are the particle size [19]. It is generally recognize that UV - Visible spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. This was tally with the parallel changes in colour which have been observed when different concentrations of neem leaf extract were used by keeping the silver nitrate solution constant. The appearance of the reddish brown colour was due to the excitation of the Surface Plasmon Resonance (SPR) of the typical of silver nanoparticles which shows the absorbance values in the visible range [19].

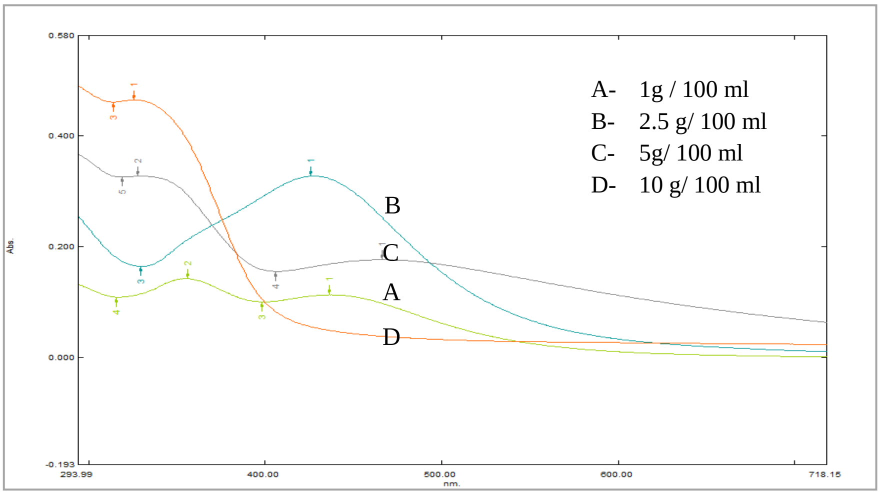

Figure 2. UV–Visible spectra recorded as a function of concentration of crude neem leaf extract in a reaction with an aqueous solution of 0.001 M $AgNO_3$

Best Mixing Ratio for the Crude Neem Leaf Extract

Further, UV–Visible spectra recorded for 2.5% crude neem leaf extract was selected for the rest of the experiments as it showed increase in intensity of absorption peak.

Initially 20 times diluted crude extract mixed with different volumes of 0.001 M silver nitrate solution resulted in absorption of 0.328 in the mixing ratio of 1:2 (Neem: $AgNO_3$) in the visible range (Figure. 3). However, when the sample directly monitored by UV-Visible spectra without any dilution, resulted distinct absorption (2.80) at 426 nm for 1:8 ratio (Figure. 4) indicating the effectiveness of the reaction ratio with the selected concentration of the crude neem extract for the formation of the silver nanoparticles.

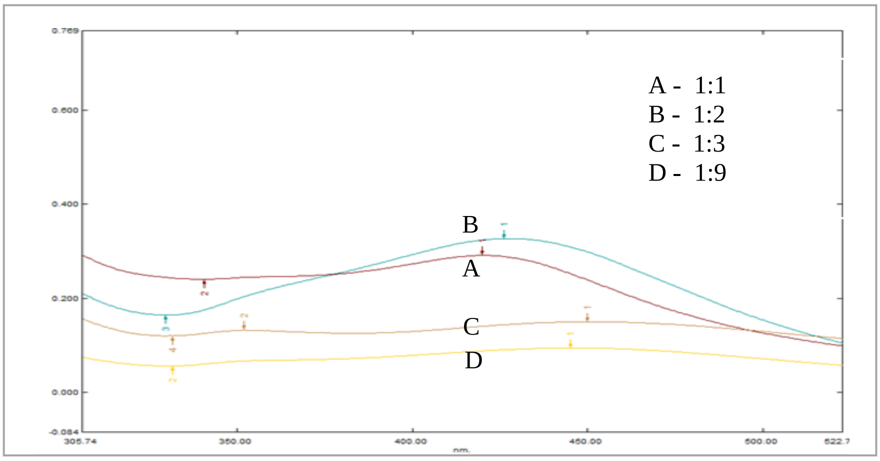

Figure 3. UV–Visible spectra recorded as a function of mixing ratio of 2.5 g/100 ml concentration of crude neem leaf extract (after diluting 20 times) with an aqueous solution of 0.001 M $AgNO_3$

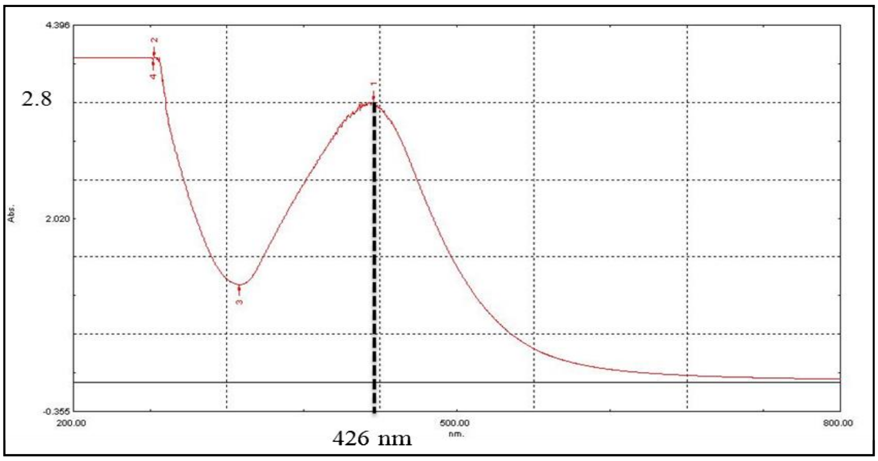

Figure 4. UV-Visible spectra of silver nanoparticles synthesized by 2.5 g/100 ml crude neem leaf extract (without any dilution) and 0.001 M $AgNO_3$ at 1:8 ratio

Best Reaction Time for the Crude Neem Leaf Extract

The UV-Visible spectrum of silver nanoparticle solution was also recorded at regular time intervals in order to study the effects of interaction time on formation of nanoparticles. There is an increase in the intensity of absorption peaks after regular intervals of time and the colour intensity increased with the duration of incubation. However, comparatively, UV- Visible spectra resulted in distinct absorption after 2 h (120 min) of mixing crude neem leaf extract (2.5g/100 ml) with 0.001 M silver nitrate solution (Figure 5).

Figure 5 represents clearly the distinct intensity in the expected visible range with the optimized parameters such as concentration of crude neem leaf extract (2.5 g/100 ml), mixing ratio at 1:8 (without any dilution) and the reaction time of 2 h. The UV-Visible spectra and visual observation could reveal the formation of silver nanoparticles in the required size and shape as reported in other studies [19]; [14].

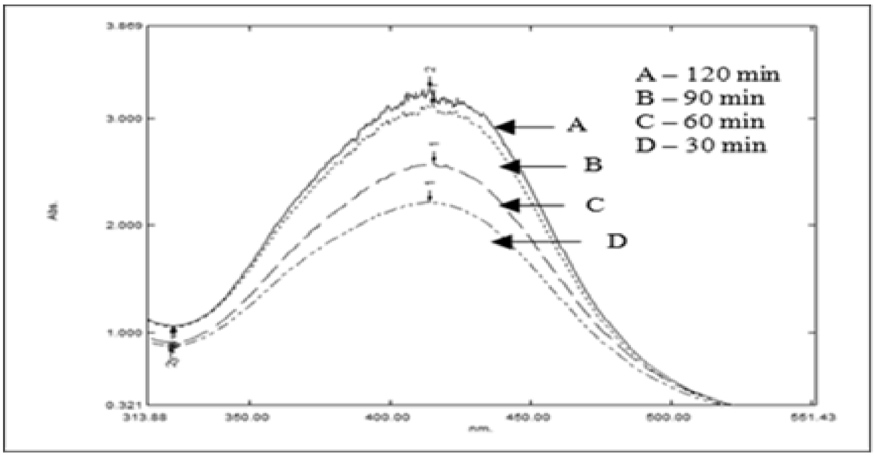

Figure 5. UV–Visible spectra recorded as a function of interaction time (30–120 min) of reaction between 2.5 g/100 ml crude neem leaf extract with an aqueous solution of 0.001 M $AgNO_3$ at 1:8 ratio

To test the next process parameter, modified neem leaf extract was selected as one type of extract. UV-Visible spectral analysis was also performed to monitor the formation of silver nanoparticles synthesized by using modified neem leaf extract as it was previously reported that neem extract made at pH 10 resulted significant antibacterial activity [15].

The UV-Visible spectrum of modified neem leaf extract showed distinct absorption in the previous studies for the concentration of 2.5g/100 ml as same as for the crude neem leaf extract (data not shown). However, the SPR absorption band was obtained at 415 nm for the ratio of 1:9 with the maximum absorption of 0.088 which was a very lower value comparatively to the crude neem leaf extract (Figure 6). In addition, SPR band was obtained even after 48 h of mixing reactants indicating the less efficiency of modified neem extract in synthesizing silver nanoparticles. This may be due to the alkaline pH values of the modified neem leaf extract. It was reported that Ag (I) ions in solution partly hydrolyze to form Bio-organic–Ag (OH)x complex on the surface of the particles and AgOH/Ag_2O colloid in the medium at alkaline pH values [19]. The amount of Ag (I) in the reaction medium may not be enough to develop significant SPR peak as same as in crude neem extract. Therefore, the reaction mixture was UV irradiated to break the synthesized AgOH/Ag_2O colloids synthesized in the modified extract. This approach may result in the breakdown of Ag_2O formed in the medium into Ag particles leading to an increase in the amounts of silver nanoparticles in the solution and eventually intensifying the maximum absorption value. UV-Visible spectra of modified neem leaf extract obtained after UV exposure at three different time intervals (15 min, 30 min and 45 min) showed distinct absorption in the expected wavelength range (Figure 7). The results showed that the initial absorption was 0.117 and further increased up to 0.148 and 0.180 after UV exposure for 15 min and 30 min, respectively. However, absorption was decreased to 0.170 after 45 min exposure to UV, indicating the required active time period for producing Ag particles to synthesize nanoparticles.

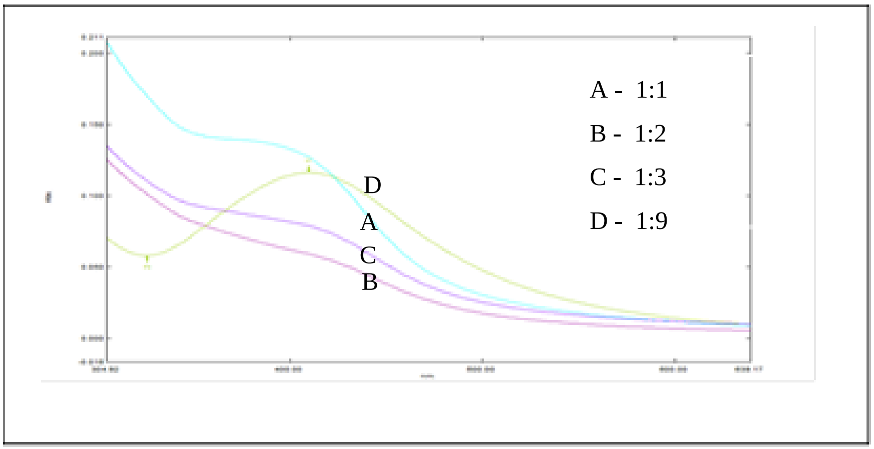

Figure 6. UV–Visible spectra recorded as a function of mixing ratio of 2.5 g/100 ml concentration of modified neem leaf extract with an aqueous solution of 0.001 M $AgNO_3$

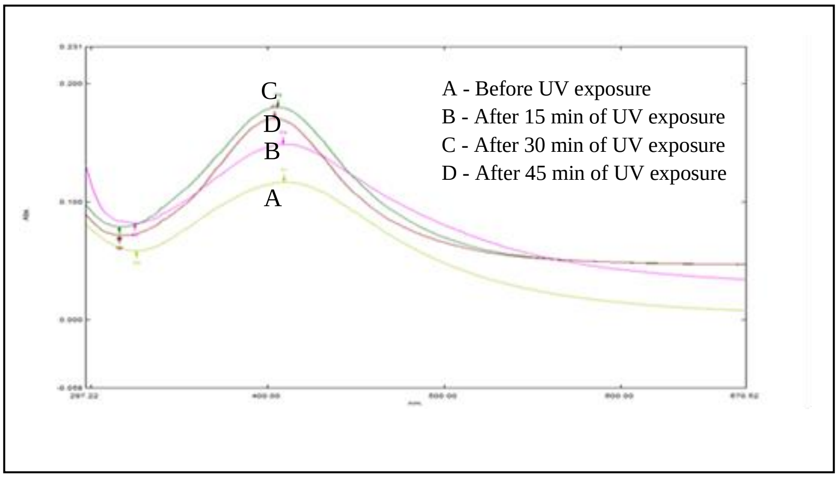

Figure 7. UV-Visible spectra recorded as a function of time of 2.5 g/100 mL modified neem leaf extract with an aqueous solution of 0.001 M silver nitrate reaction upon UV-irradiation with time

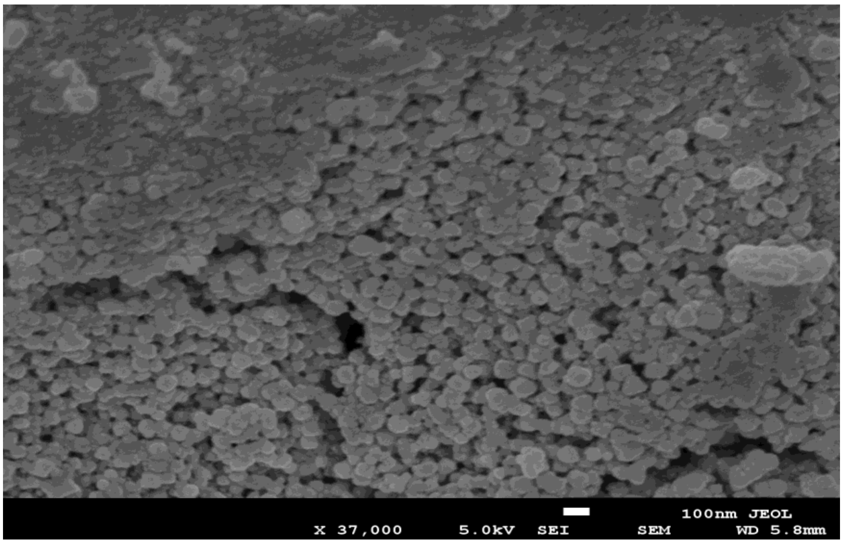

Figure 8. SEM micrograph of biosynthesized nanoparticles

The profile obtained from the SEM micrograph, see Figure 8, of the biosynthesized AgNPs by using crude neem leaf extract at ×37000 magnification showed the homogeneity of the prepared sample and indicated that silver nanoparticles are relatively spherical as well as number of aggregates with no defined morphology. The size of AgNPs was found to be in the range of 100 nm and similar result of the silver nanoparticles size was reported by using different plant extracts, including *Euphorbia hirta* leaves [20].

Conclusions

The different process parameters optimized for the crude neem leaf extract in the present study resulted in the formation of silver nanoparticles which was confirmed by both visual observations together with UV-Visible spectroscopy. SEM micrograph also revealed that the size of the nanoparticles was in the range of 100 nm. Collectively, the crude neem leaf extract of 2.5g/100 ml at the mixing ratio 1:8 with an incubation period for 120 min resulted in the distinct absorption in the expected visible range using UV-Visible spectroscopy. However, the same concentration of modified neem leaf extract at 1:9 ratio resulted the distinct absorption in the expected visible range after the exposure to UV-irradiation within 30 min. Furthermore, the fine tuning of process variables for both crude neem leaf extract and modified neem leaf extract is very important to synthesize silver nanoparticles in desirable shape and size to be applied in different fields.

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Conflicts of Interest

The authors declare no conflict of interest.

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