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Green Synthesis of Silver Nanoparticles and Its Antibacterial Activity using the Flower Extract of Senna Siamea

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Abstract

In recent science Nanotechnology is a major concern for the researchers. Nanotechnology deals with the nanoparticles having a size of 1-100 nm in one dimension due to its unique size, orientation, physical and chemical properties, which are reportedly shown to change the performance of any other material which is in contact with these particles. These particles can be synthesized by chemical, biological and physical methods. But the biological approach is the most prominent approach of preparation because this method is safer than the other methods, eco-friendly, rapid and less toxic. The green synthesis was done by using the solutions of flower extract of Senna Siamea and $AgNO_3$. Silver was chosen for this research due to its remarkable properties. A fixed ratio of plant extract to metal ion was prepared, the solution was then heated at 800W for 10 minutes, using microwave and the colour change was observed which proved the formation of nanoparticles. The nanoparticles were visualized by UV-vis spectrophotometer, which indicated that the maximum absorbance of Silver nanoparticles (AgNPs) was at 443.2 nm. Also, AgNPs were characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) and the nanoparticles obtained are spherical in shape. The synthesized silver nanoparticle has a strong bactericidal effect against Staphylococcus aurues and Eschericia coli.

Keywords: *Green synthesis, Silver nanoparticles, Senna siamea, Antibacterial activity, Microwave.*

1. INRODUCTION

Nanotechnology involves the production, manipulation and use of materials ranging in size from less than a micron to that of individual atoms [1]. Nanotechnology is often referred to as the manipulation of matter at the atomic molecular level. The word "Nano" is derived from the Greek word "nanos" which means dwarf. Nanotechnology involves the study, manipulation, creation and use of materials, devices and system typically with dimensions smaller than 100 nm [2].

Conversion of metals into nanoparticles exhibit different striking colours due to the effect of surface Plasmon Resonance [3]. Among various metals, silver nanoparticles (AgNPs) are the material of choice for researchers due to their remarkable properties such as broad spectrum antimicrobial activity, surface enhanced Raman scattering, catalytic activity, Chemical stability and Non-linear optical behaviour [4]. Broad-spectrum bactericidal and fungicidal activity of AgNPs has made them popular in the field of medicine, consumer products and agriculture. Since ancient times silver and its salts have been used as an antimicrobial agent [5]. Though its use was discontinued due to side effects [6]. Almost a decade Nano scale silver made a remarkable comeback due to its amazing properties [7-9]. AgNPs are

nanoparticles having a size range between 1nm and 100 nm in size. They are one of the widely studied nanoparticles. Different sizes and shapes can be considered and modified for various types of Applications. AgNPs are safe to use since they have low toxicity as Ag has no interaction towards living organisms during preparation [10,11].

Spherical shapes AgNPs are commonly used, but the octagon and thin sheets AgNPs also have popularity in their uses [12]. The distinctive characteristics of AgNPs make them a tool to investigate via research and evaluate their potential effectiveness, harmfulness, various shapes of AgNPs have been manufactured relying upon the demand of the Application. AgNPs have broad-spectrum antimicrobial properties so can be used in biomedical field, medical devices, textiles [13], water purifier, cosmetics, food industry [14] wound healing, therapy, DNA processing, pharmaceuticals [15] and so on.

In this study, the synthesis and characterization of silver nanoparticles /*senna siamea* (Ag/*senna siamea*) by a green method was reported. Ag-NPs were prepared using silver nitrate as silver precursor and flower extract of senna siamea as a reducing agent and stabilizer. The antibacterial effect of Ag/*senna siamea* was evaluated against two pathogenic bacteria, including *Escherichia coli* (Gram negative) and *Staphylococcus aurues* (Gram positive) using the agar disc diffusion method.

There have been several reports on the synthesis of AgNPs using plants such as *Basella alba*, *Helianthus anus*, *Saccharum* officinarum, *Oryza sativa*, Sorghum bicolour, *Zea mays* [16], Aloe vera [17], *Medicago sativa*(Alfafa) [18], *Capsicum annuum* [19], *Magnolia kobus* [20], *Cinnamon camphora* leaf [21], *Geranium sp*.[22], Milk-thistle plant (*silybum marianum*) seed extract [23], Amazon fruit [24] and carica papaya peel extract [25] for pharmaceutical and biological applications. A synthesis using a methanolic extract of *Eucalyptus hybrida* leaves was reported [26], another study from *Vitex negundo L*. leaf extract in water solution with heat treatment was also reported [27].

2. EXPERIMENTAL

2.1 . Sample Collection

The flowers of *Senna siamea* were collected from Umaru Musa Yaradua University Katsina. All reagents used were prepared using double distilled water. The reagent used was of analytical grade.

2.2 . Extract Preparation

Senna flowers were washed and dried, the dried flowers were then ground into powder and kept at room temperature till further analyses. The finely ground flowers (20 g) were extracted with distilled water. After filtration with Whatman filter paper, the residue was re-extracted, the extract was kept at a very cool temperature until used.

Figure 1. *Flowers of senna siamea.*

2.3 . Synthesis of Ag/*Senna Siamea*

10 mL of extract was added to 90 mL of 0.005 M $AgNO_3$, the solution was then heated using microwave at 800W for 10 minutes, and gradually silver nanoparticles were formed during the incubation period.

2.4 . Evaluation of Antibacterial Activity

This was carried out using the disc diffusion method. The test organisms were obtained from microbiology lab, UMYU katsina. The isolates were sub cultured onto Nutrient agar prepared according to manufacturer's instructions. They were

incubated for 24 hrs at 37 °C in a laboratory incubator.

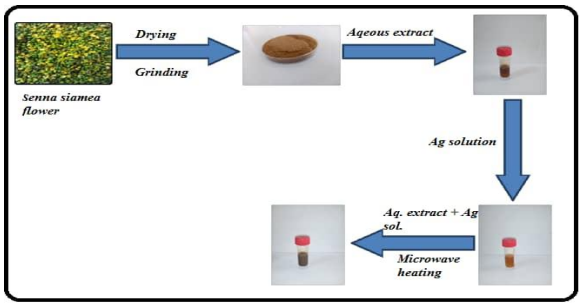

Figure 2. *Flow chart for the formation of silver nanoparticles.*

The growth obtained was used to prepare standard inoculum of both *E.coli* and *S.aureus*. 5ml of distilled water was introduced into a sterile test tube, a sterilized wire loop was used to scoop colonies from the plates. The colonies were emulsified into the distilled water; this was continued until the distilled water has a turbidity matching the 0.5 McFarland standard provided. This was assumed to contain approximately 1.5×10^6 colony forming units.

The antimicrobial assay was carried out on Nutrient agar from the standardized inoculum, 100 microlitres corresponding to 0.1 mL were spread over the nutrient agar plates and allowed to settle for 30 mins. The spreads was done using a sterile cotton swab stick. Different concentrations of the nanoparticle namely; 250, 125, 62.5 and 31.25 mg/ml were prepared using dilution method. A hole puncher was used to punch holes into a Whatman filter paper to obtain discs roughly 6 mm in diameter, the punched discs were wrapped in aluminium foil and sterilized by autoclaving at 121 °C for 15mins. The sterilized discs were dipped into the tubes containing various concentrations of the nanoparticle, a sterilized forceps was used to pick the discs and place them over the plates containing the spread inocula. Two concentrations were placed on every plate. Ciprofloxacin of 50 mg was used as positive control.

Minimum inhibitory concentration (MIC) was determined by preparing the Nutrient broth according to manufacturer's instruction, concentrations of the nanoparticle ranging from 15.63, 7.81, 3.91 to 1.95 were prepared, 5 mLs of the prepared nutrient broth together with 0.5 mL of the prepared concentrations of the nanoparticle and 50 µl of the McFarland standard were put into the test tube using a syringe and micropipette. The tubes were incubated at 37 °C for 24hrs and subsequently turbidity or lack thereof was checked for. The tube containing the lowest concentration that shows no turbidity was regarded as the MIC.

3. CHARACTERIZATION METHODS AND INSTRUMENTS

3.1. UV-vis Analysis

The optical property of Ag-NPs was determined by UV-Vis spectrophotometer. After the addition of $AgNO_3$ to the plant extract, the spectra was taken at the range of 400-800 nm.

3. 2. SEM Analysis

The morphological features of synthesized silver nanoparticles from flower extract of *Senna siamea* were studied by Scanning Electron Microscope. After addition of $AgNO_3$ and heating, the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized by SEM at an accelerating voltage of 15KV.

3. 3. FTIR Analysis

The chemical composition of the synthesized silver nanoparticles was studied using FTIR Spectrometer. The solutions were dried and the dried powders were characterized in the range of 4000-650cm^{-1}.

4. RESULTS AND DISCUSSIONS

Green synthesized silver nanoparticles using 0.005M of $AgNO_3$ is shown in Fig 3

below. The fresh suspension of *senna siamea* flowers was brown in colour. However, after the addition of $AgNO_3$ and heating at 800W, the emulsion turned dark brown.

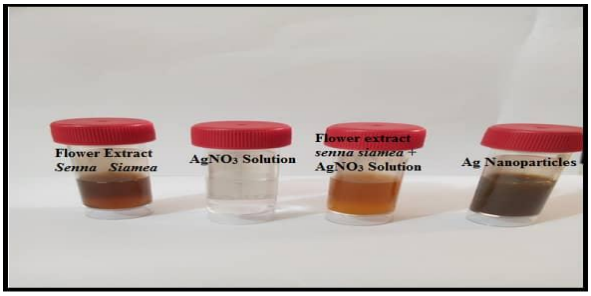

Figure 3. *Colour changes of silver nanoparticles.*

Reduction of silver ions into silver nanoparticles during exposure to flower extract was observed as a result of the colour change. The colour change is due to the Surface Plasmon resonance phenol-menon. The metal nanoparticles have free electrons which give the SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles are observed around 443.2 nm as shown in figure 4 below.

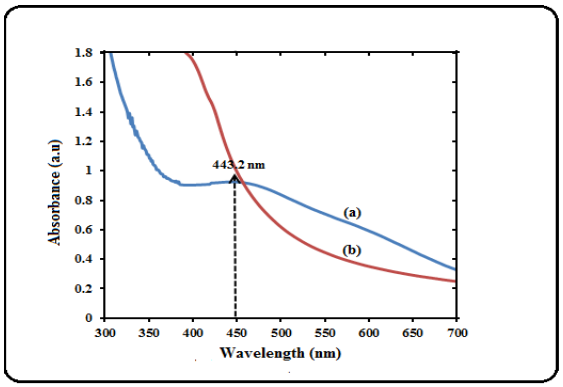

Figure 4. *UV spectra of silver nanoparticles, synthesized silver nanoparticle (a) and flower extract of senna siamea (b).*

SEM provided further insight into the morphology and size details of the silver nanoparticles. The result shows that the particles are of spherical shape as shown in figure 5 below.

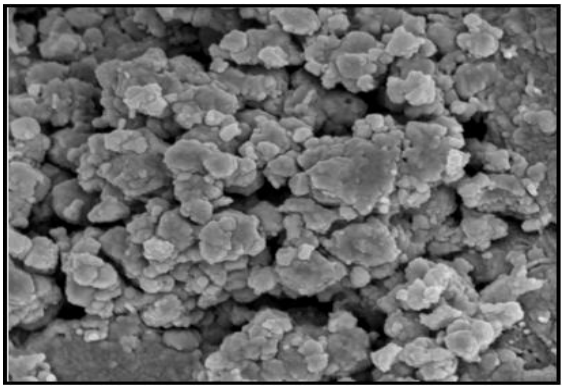

Figure 5. *SEM image of silver nanoparticles*

FTIR measurements are carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles shows the band around 3298cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1730cm^{-1} shows a bond stretch for C-H bond, peak around 1629cm^{-1} showed the bond stretch for N-H. Whereas the bond stretch for Ag-NPs were found around 1033cm^{-1}.Therefore the synthesized silver nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of amines. Carbonyl groups proved that flavanones absorbed on the surface of metal nanoparticles as shown in figure 6.

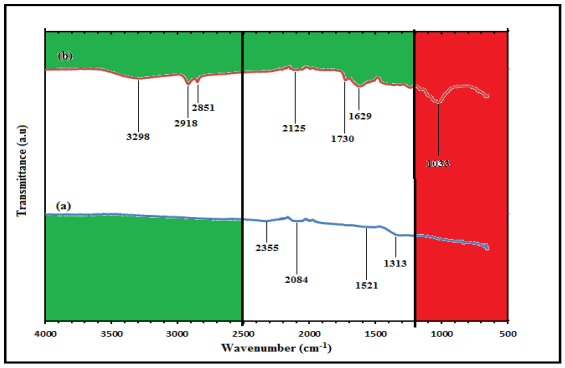

Figure 6. *FTIR result for silver nanoparticles, flower extract of senna siamea (a) and synthesized silver nanoparticle (b).*

The antibacterial activity of the silver nanoparticles synthesized using flower extract of *senna siamea* was investigated against the Gram positive bacteria staphylococcus aureus and against the Gram negative bacterial *Escherichia coli*. The images showing the antibacterial activity are shown in figure 7 and the values of zone of inhibition observed are given in table 1 below. The antibacterial activity of AgNPs was observed to increase with increase dosage. It has already been reported that the bacterial activity of nanoparticles depends on size and dose and they usually show more activity against Gram negative than Gram positive as reported by [28]. The flower extract did not show any antibacterial activity at the concentration level used in this study. Many possible mechanisms have been proposed for explaining the antibacterial activity of silver. It is believed that Ag interacts with the cell walls thereby blocking respiration and causing death [28].

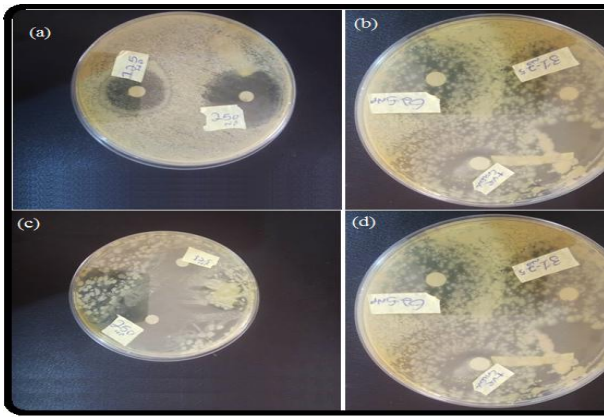

Figure 7. *Inhibition zones, images of AgNPs at different concentrations 250, 125, 62.5, and 31.25 mg/ml against Staphylococcus aurues (a-b) and Escherichia coli (c-d).*

The minimum inhibitory concentration of synthesized Ag/*senna siamea* nanoparticle against *E.coli* and *S.aurues* is 15.63 and 7.81 respectively.

5. CONCLUSION

The rapid biological synthesis of silver nanoparticles using the flower extract of *Senna siamea* provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles.

Table 1. *Average zones of inhibition of synthesized Ag/senna siamea nanoparticles.*

Bacteria	**250 (mg/ml)**	**125 (mg/ml)**	**62.5 (mg/ml)**	**31.25 (mg/ml)**	**Positive control (ciprofloxacin)**
E. coli	32.5±0.05	29±0.03	28.5±0.09	12.5±0.02	39.5±0.08
S. aurues	30.5±0.07	31.5±0.09	23.5±0.05	16±0.07	26±0.09

Table 2. *Minimum inhibitory concentrations.*

Bacteria	**15.63 (mg/ml)**	**7.81 (mg/ml)**	**3.91 (mg/ml)**	**1.95 (mg/ml)**
E. coli	-	+	+	+
S. aurues	-	-	+	+

The synthesized nanoparticles were of spherical shape and have a wavelength of 443.2 nm. The nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols and ketones which were found from the characterization using UV-vis spectrophotometer, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) techniques. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness compatibility for medical and pharmaceutical applications as well as large scale commercial production. The synthesized silver nanoparticles shows more antibacterial activity towards *E.coli* than *S.aurues*.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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