Advances in Bioscience and Bioengineering
2021; 9(4): 124-129
http://www.sciencepublishinggroup.com/j/abb
doi: 10.11648/j.abb.20210904.14
ISSN: 2330-4154 (Print); ISSN: 2330-4162 (Online)

Green Synthesis of Silver Nanoparticles Using *Euphorbia hirta* Leaf Extract and the Determination of Their Antimicrobial Activity

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To cite this article:
Egbunu Iganya Edith, Philip Felix Uzor. Green Synthesis of Silver Nanoparticles Using *Euphorbia hirta* Leaf Extract and the Determination of Their Antimicrobial Activity. *Advances in Bioscience and Bioengineering.* Vol. 9, No. 4, 2021, pp. 124-129. doi: 10.11648/j.abb.20210904.14

Received: November 25, 2021; **Accepted**: December 14, 2021; **Published**: December 24, 2021

Abstract: Nanotechnology is a fast-growing field of science. Nanoparticles get much attention due to their unique physicochemical, optical and thermal activities. Silver nanoparticles have been used in experiments to treat infectious diseases. The goal of the research was to make silver nanoparticles using *Euphorbia hirta* extract, physically characterize the nanoparticles obtained and, to evaluate silver nanoparticles' antibacterial properties. The leaf extract of *E. hirta* (asthma weed) was used for the reduction of 1 mM silver nitrate ($AgNO_3$) solution to silver nanoparticles (SNPs). SNPs were made by combining 50 mL of aqueous plant extract with 250 mL of $AgNO_3$ solution to make SNPs. The mixture was monitored for two hours. The synthesized SNPs were characterized by UV-Vis, FTIR spectroscopy and particle size. The antimicrobial activity of the SNPs was tested against *Escherichia. coli, Pseudomonas. aeruginosa, Salmonella. typhi, Bacillus. subtilis* and *Candida. albicans*. The reaction medium's hue shifted from yellow to brown. The results of the UV-vis analysis of the particles showed that at 430 nm the particles had the maximum absorption (λ max) within 2 hours. The FTIR identified carboxylic acid and other functional groups. The polydispersity index (PDI) and Z-average particle size were found to be 0.426 and 274 nm, respectively. The results of the antimicrobial studies showed sufficient growth inhibition of the bacteria by the SNPs the minimum inhibitory concentration (MIC) ranging from 7 µg-10 µg. It was concluded that SNPs were synthesized using *E. hirta* leaf extract. The synthesized SNPs possess good activity against pathogenic microorganisms.

Keywords: Nanoparticles, Synthesis, Extraction, Antimicrobial, Nanotechnology

1. Introduction

The plant *Euphorbia hirta* originates from the family Euphorbiaceae; it is commonly known as asthma herb [1]. The greenish or reddish leaves (5 cm long) have oppositely arranged lanceolate clusters of flowers-like appearance [2]. The stem and leaves produce [3] white or milky juice when cut [4] The leaves are used to treat cough, asthma, worms, and vomiting. It has deworming properties, and it is also eaten as vegetables. The white latex is used as eye drops to cure conjunctivitis. Paste of leaf is applied externally on the place of scorpion bite. *Euphorbia hirta*. latex is rubbed on swellings, piles, and boils, and the root decoction is applied for snake bites, sores, wounds and is advantageous for nursing mothers with insufficient milk. The whole plant can be stipulated as an antidote; it is believed to be hemostatic, sedative, and soporific [5].

The manufacture of particles with at least one fraction in the range of 1–100 nm, resulting in a high surface-to-volume ratio, is known as nanotechnology [6]. Despite the diversity of metals found in nature, only a small percentage of them, such as gold, silver, palladium, and platinum, are widely synthesized in nanostructured form [7]. Silver nanoparticles have received a lot of attention since they can be utilized in a variety of applications, including pharmaceutics, agriculture, water detoxification, air filtration, textile industries, and as an oxidation catalyst [8]. Furthermore, one of their most important qualities is their antibacterial action against a wide spectrum of microorganisms without causing harm in animal cells. [9].

Diverse parts of plants have been used to synthesize silver nanoparticles (SNPs) including [10], barks [11], seeds [12], etc. Different research papers have reported the antimicrobial activity of SNPs. The mechanism of antibacterial activity of SNPs is a source of debate that is currently poorly understood. There are, nevertheless, several assumptions and theories [13]. The goal of the study was to see how antimicrobial silver nanoparticles made from *Euphorbia hirta* extract performed.

2. Methodology

2.1. Preparation of Plant Extract

The leaves of the E. hirta plant were obtained from Heipang in Barkin Ladi LGA, Plateau State, Nigerian in October 2020. Following that, 20 g of powdered material was placed in 400 ml of distilled water, and the mixture was heated at 100°C for 30 minutes. Whatman filter paper no. 1 was used to filter the extract after it had been cooled.

2.2. Green Synthesis of Silver Nanoparticles

Silver nitrate (0.1698 g) was weighed accurately with an analytical weighing balance and dissolved with distilled water in a 50 ml beaker; it was then transferred to a 1 L volumetric flask where the volume of the solution was made up to the 1 L mark of the volumetric flask to make 1mM solution. The SNPs solution provided the silver ion for the reaction. The plant extract (50 ml) was mixed with 250 ml of $AgNO_3$ solution 1 mM. The reaction was incubated at a temperature of 27°C in the dark to avoid photochemical activation of $AgNO_3$. Colour change to dark brown was taken as an indication of synthesis of SNPs. The obtained solution was centrifuged for 30 minutes at 5000 rpm. To remove silver ions and seed extract residue, the pellet containing silver nanoparticles was washed 3–4 times with distilled water.

2.3. Ultraviolet-visible Spectroscopy (UV-Vis)

The extracted SNPs were analyzed by scanning under a wavelength ranging from 300-800 nm using the UV-VIS spectrophotometer (JENWAY, 6705) at 30 minutes for 2 hours.

2.4. Particle Size Determination

Dynamic light scattering was used to figure out the particle size (D. L. S.). In addition, the polydispersity index and Z-average were recorded. The instrument used for the analysis was the (Malvern Nano ZS7.01).

2.5. Fourier Transformed Infrared Spectroscopy (FTIR)

The FTIR analysis of the SNPs was done using the FTIR-8400 S spectrophotometer system.

2.6. Antimicrobial Analysis

Agar diffusion method was adopted for the antimicrobial assay. The silver nanoparticles extracted were diluted at ten (10) different concentrations (1, 2, 3…10 µg/ml). The control drugs used were (fluconazole and ciprofloxacin). The agar was prepared and autoclaved and poured into 13 agar plates; the different concentrations of the SNPs two standard drugs and the solvent (control) were added to the 13 different plates, one sample per plate. Then, the plates were allowed to solidify; Each plate was marked to divide into the different microorganisms; the different microorganisms were indicated by 1, 2, 3, 4, 5, 6 as six organisms were used (*S. typhi, E. coli, B. subtilis, S. aureus, C. albicans,* and *A. niger*). Each microorganism was streaked on the surface of each division of the agar plate, one microorganism per division. The plates were then incubated for 24 hours.

3. Results

3.1. Visual Observation and UV-visible Spectroscopy

The reaction changed the color of the reaction mixture from yellowish-brown to dark brown, as shown in Figure 1, indicating that the Ag+ ion was reduced.

Figure 1. *Change in color after reducing Ag^+ to silver nanoparticle by using Euphorbia hirta leaf extract. Keys:(A) at 0 minutes, (B) at 30 minutes, (C) at 1 hour.*

The ultraviolet-visible spectroscopy graph at 0 hour Figure 2 shows the initial onset of reaction; it can be seen that for the initial onset, a valley was observed before a sudden peak in the reaction at about 430 nm this is also reflected in Figure 8 which shows the combined graph of the reaction from 0 to 2.5 h.

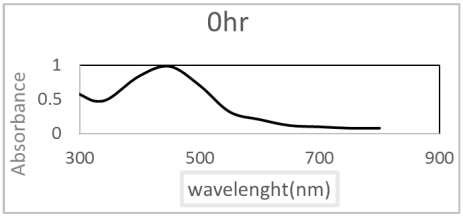

Figure 2. *UV-Vis Spectroscopy at 0 hour.*

In Figures 3-7, it can be seen that absorbance increased with time (0.5-2.5h) almost the same wavelength as in Figure 2; this indicated an increase in the amount of the SNPs synthesized with an increase in time.

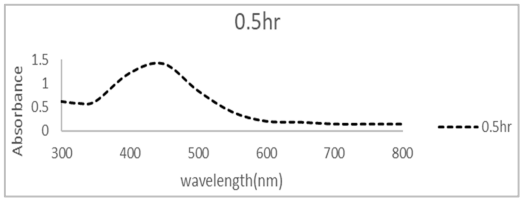

Figure 3. *UV-Vis spectroscopy at 0.5 hour.*

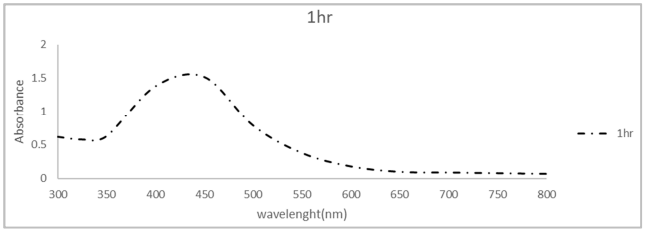

Figure 4. *UV-Vis spectroscopy at 1 hour.*

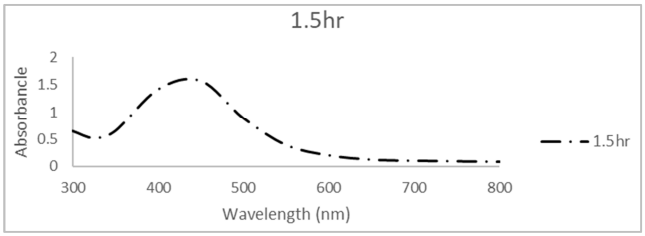

Figure 5. *UV-Vis Spectroscopy at 1.5 hour.*

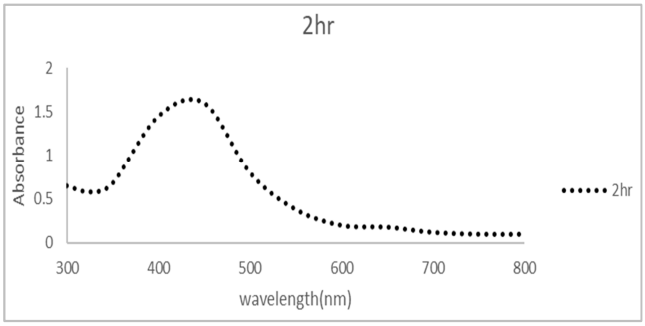

Figure 6. *UV-Vis Spectroscopy at 2 hours.*

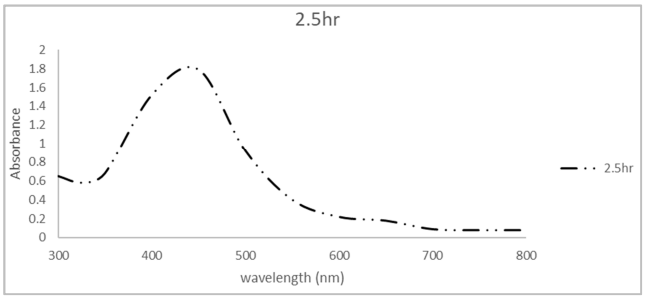

Figure 7. *UV-Vis spectroscopy at 2.5 hours.*

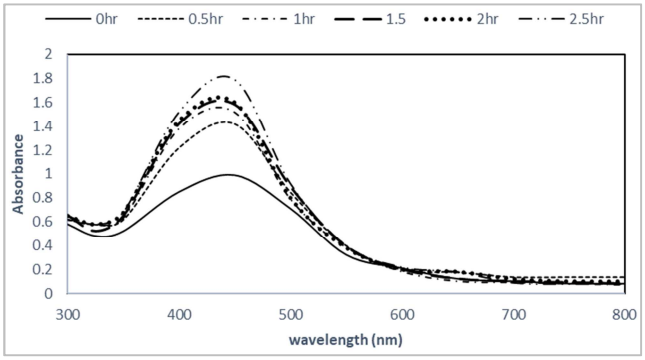

Figure 8. *UV-vis absorbance characteristics displayed by AgNPs of E. hirta leaf extract at different time interval.*

3.2. Particle Size Analysis

The Z-average of the silver nanoparticles was 274.6 nm (diameter), and the polydispersity index was 0.426, according to dynamic light scattering, commonly known as photon correlation spectroscopy analysis. Figure 9.

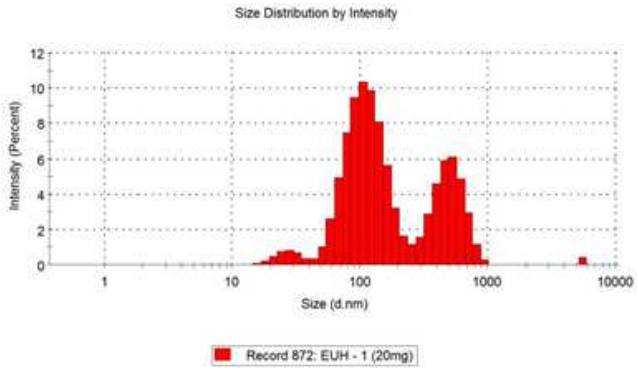

Figure 9. *Particle size distribution by intensity for synthesized silver nanoparticles (SNPs).*

Table 1. *Particle size distribution by intensity.*

	Size (d.nm)	Intensity (%)
Peak 1	116.4	68.4
Peak 2	502.6	31.0
Peak 3	28.65	3.7
Z-average (d.nm)	274.6	
PDI	0.426	
Particle size range	<100nm	

From the analysis carried out, it can be noted that the average particle size of the E. hirta silver nanoparticle extract was 274.6 d.nm (Z-average). From Table 1 above, it can be observed that the intensity of peak 1 was 68%, and in the graph of Figure 9, the highest intensity of the particles was within a range of 90-120 nm (diameter), the aggregation of the particles over time resulted in a subsequent increase in particle size.

The numerical value of PDI for the extract was 0.426, which is within the range for nanoparticles indicating that even though the particles are not evenly distributed, they have some form of uniformity in distribution.

3.3. Results of FTIR

The results of FTIR analysis are presented in Table 2.

The study was conducted to discover the various functional groups found in SNPs and their role in the stability and production of silver nanoparticles. Several peaks indicating the complex nature of the biological material. The stretching vibrations of the C-O and OH link carboxylic acid, alcohol, 2345.52 nitrile, N-O nitro compound, 3441.12 N-H amine group, Si-OH silanol are ascribed to the bands occurring at 1103.32, 3178.79, 3294.53, 3510.56, 3626.29, and 3772.89 cm-1, respectively.

Table 2. *FTIR analysis of silver nanoparticles obtained using E. hirta leaf extract.*

Peak number	Absorption frequency (cm^{-1})	Area	Intensity	Possible bond	Possible functional group
1.	933.58	0.547	1.403	C-H	Alkene
2.	1003.02	0.035	0.338	C-H	Alkene
3.	1103.32	0.048	0.328	C-O	Alcohol, carboxylic acid, ether
4.	1450.52	0.076	0.645	C=C	Alkene
5.	1527.67	0.054	0.209	C=C	Alkene
6.	1689.7	0.695	1.564	N-O	Nitro compound
7.	1928.88	0.02	0.168	R═N═C═S	Isothiocyanates
8.	2252.93	0.043	0.147	C-H	Alkene
9.	2345.52	0.632	1.991	C≡N	Nitrile
10.	2723.58	0.052	0.359	C-H	Alkane
11.	2839.31	0.107	0.379	C-H	Alkene
12.	3178.79	0.226	0.634	O-H	Carboxylic group
13.	3294.53	0.225	0.535	O-H	Carboxylic group, H- bonded
14.	3441.12	0.188	0.976	N-H	Amines
15.	3510.56	0.076	0.476	O-H	Carboxylic acid
16.	3626.29	0.376	1.716	O-H	Alcohol
17.	3772.89	0.093	0.5	O-H	Alcohol, carboxylic acid, free hydroxyl group
18.	3865.48	0.852	2.649	Si-OH	Silanol
19.	3934.92	0.11	0.682	Si-OH	Silanol

3.4. Antimicrobial Assay

The MIC of antimicrobial drugs against various bacteria was determined using the Agar dilution method. S. typhi had a MIC of 10 g/ml, E. coli 9 g/ml, B. subtilis 7 g/ml, S. aureus 9 g/ml, and C. albicans 9 g/ml after 24 hours of incubation.

4. Discussion

SNPs have been synthesized with different varieties of plants such as *Azadirachta indica, Annona muricata, Origanum vulgare*, e. t. c. The creation of silver nanoparticles began with the addition of *Euphorbia hirta* leaf extract to 1 mM silver nitrate ($AgNO_3$). The gradual shift in color of the mixture from yellowish to reddish-brown, as depicted in Figure 1, indicated the production of SNPs. Surface Plasmon (i.e., quantum of plasma oscillation) vibration causes the color change, which is an optical feature specific to noble metals. [14]. To get a decent outcome, several parameters had to be tuned, including the concentration of $AgNO_3$, *E. hirta* leaf extract, and time, all of which have been identified as factors impacting SNP production. It was also observed in other plant extracts that heating of the reaction mixture also increased the yield of silver nanoparticles.

UV-Vis spectroscopy was used to further examine the sample at various time intervals. The samples had valleys within 300-400 nm wavelength and peaks at 400-500 nm wavelength; the maximum wavelength from the combined graph see Figure 8 above was 430 nm which is characteristic of SNPs [15]. The curve in Figure 8 showed that with an increase in incubation time there was an increase in absorbance which was similar to previous reports. [13], The reaction lasted for 2.5 hours, the same time interval was seen in earlier study [16]. A range of factors could have affected the UV-Vis absorption characteristics of the extract; these could include; solvent, concentration, pH, temperature.

By modifying the position (max) and intensity (max) of the chromophore's absorption peaks in the compound, uncontrolled alterations in these factors can cause inaccuracies. These parameters were adequately controlled in order to obtain a meaningful and quantitative result [17]. A broad peak at a higher wavelength usually implies an increase in particle size, whereas a thin line at a shorter wavelength usually suggests a decrease in particle size. Other studies have shown that raising the percentage of plant extract can greatly influence the size and size distribution of nanoparticles [18].

A broad peak at a longer wavelength usually indicates an increase in particle size, whereas a thin line at a shorter wavelength usually indicates a decrease. Other research has found that increasing the proportion of plant extract in nanoparticles has a significant impact on their size and size dispersion [19].

The presence of different primary functional groups in the SNPs and their likely participation in the synthesis and stability of SNPs was detected using Fourier transform infrared spectroscopy (FTIR). The functional groups observed are comparable to those seen in prior investigations [20]. The existence of O–H stretch and hydrogen-bonded groups in alcohol, phenolic, or water molecules in the extract can be seen in the broad stretch at 3294 cm^{-1} [21]. The coordination of SNPs with –OH and C=O groups may be responsible for the stability and capping agent of synthesized SNPs. It's also possible that the reduction process is caused by the presence of phenolic and flavonoid group molecules; the phenol present has disinfectant capabilities [22]. Antioxidant, anti-allergic, anti-cancer, anti-inflammatory, and antiviral effects are all found in flavonoids. For example, the flavonoid quercetin has been shown to help with asthma, hay fever, and sinusitis symptoms [23].

Antibacterial testing on the SNPs revealed impressive antimicrobial activity, however it was lower than that of the control antibiotics (gentamicin and ofloxacin) utilized in Table 2 above. Biogenic SNPs have previously been shown to be more effective antibacterial agents than chemically produced SNPs [24]. In the results of the P. aeruginosa and E. coli were found to have the highest sensitivity to the synthesized SNPs, while C. albicans was more resistant to the synthesized SNPs

[14]. The minimum inhibitory concentration (MIC) of SNPs against *S. typhi, E. coli, B. subtilis, S. aureus, C. albicans, and A. niger* was determined after the antibacterial activity of produced AgNPs was confirmed using a disc diffusion experiment. The MIC of antimicrobial drugs against diverse bacteria was determined using the agar dilution method. After a 24-hour incubation period, the MIC was noted, *S. typhi* had a MIC of 10µg/ml, *E. coli* 9µg/ml, *B. subtilis* 7µg/ml, *S. aureus* 9µg/ml and *C. albicans* 9µg/ml.

5. Conclusion

Based on the study carried out above *E. hirta* leaf extract was shown to be an efficient reducing agent for the synthesis of SNPs; the SNPs were made utilizing *E. hirta* leaf extract.,. The synthesized SNPs showed remarkable stability. The nanoparticles showed profound antimicrobial activity that could be used to develop antimicrobial drugs. The procedure for producing silver nanoparticles was found to be easy, quick, eco-friendly, and non-toxic.

6. Recommendation

Further research can be carried out on the silver nanoparticles to make them useable in the formulation of creams or ointments for the treatment of infections caused by sensitive organisms.

Acknowledgements

We thank the Madonna University Pharmaceutical Chemistry and the Pharmaceutical Microbiology laboratory staff for providing us with the necessary apparatus and equipment and their support during the research.

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