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Green Synthesis of Silver Nanoparticles Using *Cannabis sativa* Extracts and Their Anti-Bacterial Activity

Sujata Mandal[1], Sreekar B. Marpu[2], Roxana Hughes[3], Mohammad A. Omary[2*], Sheldon Q. Shi[1*]

[1]Department of Mechanical Engineering, University of North Texas, Denton, TX, USA
[2]Department of Chemistry, University of North Texas, Denton, TX, USA
[3]Department of Biological Sciences, University of North Texas, Denton, TX, USA
Email: *sheldon.shi@unt.edu, *omary@unt.edu

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Abstract

A procedure for the green synthesis of silver nanoparticles (AgNPs) using *Cannabis sativa* (hemp plant) as a stabilizing media was developed and antibacterial activity was tested. Within 30 minutes of heating the mixture of silver nitrate and hemp extract, the formation of silver nanoparticles took place under the complete absence of a chemical reducing or an additional stabilizing agent. The so-formed AgNPs were characterized using different optical spectroscopy and electron microscopy techniques. The initial formation of AgNPs was established from UV-Vis data based on surface plasmon resonance (SPR) of AgNPs at ~417 nm. The exact size, shape, and elemental composition of AgNPs were established from ESEM images and EDS data. The antibacterial activity of these nanoparticles was studied on Gram-positive *Staphylococcus aureus*, and Gram-negative *Escherichia coli* following Disk diffusion and Minimum Inhibitory Concentration (MIC) tests. Results showed that the biosynthesis of silver nanoparticles using hemp extract could be a simple, inexpensive, and biocompatible method.

Keywords

Hemp, Silver Nanoparticles, Green Synthesis, Pathogens

1. Introduction

Water contamination is one of the most persistent issues for public health. Waterborne pathogens have a devastating effect on human health. Multidrug-resistant bacteria, also known as superbugs, are being found in wastewater treatment plants

[1]. A report by the United Nations, Interagency Coordination Group (2019) estimated that by 2030, the antimicrobial-resistant superbugs not only claimed the lives of 10 million people but would also destabilize the global economy [2]. As the demand for fresh drinking water is increasing, nanotechnology is gaining tremendous impetus in the present century to provide safe and affordable drinking water. The goal of the present research is to remove emerging pathogens from water without intensive use of harsh chemicals or the production of toxic byproducts. Nanomaterials can be synthesized by different techniques such as precipitation, chemical reduction, thermal decomposition, photochemistry, and microwave-assisted techniques [3] [4]. However, the major limitation for many of these techniques is the usage of strong reducing and stabilizing chemical reagents that pose a serious threat to the environment and human health [3]. In recent years, environmental and health concerns have stimulated researchers to devise novel and biological approaches to synthesizing nanomaterials using biological systems like microorganisms and plants, known as "green chemistry", approaches. In our previous efforts, we have shown the formation of size-tunable silver nanoparticles by photochemical reduction of Ag(I) salts in presence of biologically benign polymer media (no chemical reducing or nanoparticle growth-assisting reagents), as another manifestation of "green chemistry" [5].

The use of parts of the whole plant for nanoparticle synthesis offers exciting and potential advantages with a great capacity for investigation. Silver is one of the most widely used metals due to its inhibitory effect on bacteria and microorganisms by producing reactive oxygen species and thereby damaging the cell organelles [6] [7] [8]. Silver nanoparticles are considered a novel and probable substitute to standard antibiotic drugs as these pathogenic bacteria exhibit an increased multidrug resistance property.

Biosynthesis of silver nanoparticles using the abundant and sustainable plant resources is suitable for accomplishing the great demand for "biocompatible and green-synthesized nanoparticles", especially for the applications in biomedical and environmental areas. Hydroxyl and carboxyl groups found abundantly in the green plant materials act as a good source of reducing agents in the formation of metal nanoparticles, whereas the strong interaction between biomolecules and nanoparticles contributes to the excellent stability of the AgNPs [9] [10]. Moreover, the presence of phytonutrients in green resources plays an active role as a reducing and stabilizing agent for metal nanoparticles [11] [12].

Many researchers have reported the synthesis of silver nanoparticles using various natural products, e.g. green tea (*Camellia sinensis*) [13] [14], leaf broth of *Acalypha indica*, neem (*Azadirachta indica*) leaf extract [15] Aloe vera leaf extract [16] [17] [18], starch [19], and lemongrass leaves extract [20]. Hemp (*Cannabis sativa*) mainly contains cellulose, hemicellulose, and lignin, and has been widely cultivated in many tropical countries for its fiber content [21]. Hemp has been reported as a good source of food, oil, and fiber. It is also noted

as a good source of pharmaceutical ingredient [22], as it contains various bioactive substances like cannabinoids, terpenes, ketones, fatty acids, and phenolic compounds demonstrated for their antibacterial, antifungal, anti-inflammatory, and anticancer properties [23] [24] [25]. Contrasting to the prior work in this field, in the present study, an eco-friendly method for the rapid synthesis of silver nanoparticles (AgNPs) using the hemp plant (*Cannabis sativa*) has been developed. No toxic chemicals were used as reducing agents or stabilizing agents during the synthetic protocol. The AgNPs were found to exhibit antibacterial efficacy against selective gram positive and gram-negative bacteria.

2. Experimental

2.1. Materials and Methods

Dry hemp hurd (HH) was collected locally. Silver nitrate was purchased from Sigma-Aldrich (St. Louis, MO, USA), Luria Agar Base was obtained from (Santa Maria CA USA) and deionized water (18.2 $M\Omega cm^{-1}$) was used during all experiments. The HH helps in reducing Ag(I) to Ag(0) and stabilizing the so formed Ag(0).

2.2. Preparation of Hemp Hurdbased Carbon Extract (HHC)

Two grams of HH were soaked in 100 mL of deionized water and boiled at 60˚C for 1 hour. The solution was then filtered. The filtrate, henceforth termed as hemp hurd based carbon extract (HHC) and was stored at 4˚C to carry out further experiments. Figure 1 showed the changes in physical color both for the concentrated and the dilute (after the addition of silver nitrate) HHC solutions.

2.3. Biosynthesis of Silver Nanoparticles

A 5 mL of aliquot (HHC) was added dropwise into 20 mL of 1 mM and 5 mM silver nitrate solutions respectively and mixed through constant magnetic stirring at temperature 70˚C for 30 minutes. The effect of $AgNO_3$ concentration and stirring time on the formation of AgNPs was analyzed. The formation of silver nanoparticles within the HHC media was confirmed from Figure 1. The colorless HHC solution exhibited a distinct brown color due to the formation of silver nanoparticles.

Figure 1. Daylight picture of HHC, HHC-silver salt, and HHC-AgNPs sample. **A**—Concentrated HH-2%, **A′**—HHC diluted with $AgNO_3$ (1 mM), **B** and **C**—HHC stabilized AgNPs sample at 15 minutes and 30 minutes of heating.

2.4. Characterization of the Synthesized Silver Nanoparticles (AgNPs)

The successful formation of the silver nanoparticles was confirmed from the surface plasmon resonance (SPR) of AgNPs obtained using a UV-Vis spectrophotometer (Perkin-Elmer Lambda-900). The change in the physical color of the samples before and after the formation of AgNPs in HHC solution can be noticed in Figure 1. The SPR peaks were documented for two different concentrations (1 mM-HFN1 and 5 mM-HFN2) of silver nanoparticles at different stirring times of (15 minutes and 30 minutes respectively) The samples were labeled as HFN1-15, HFN1-30, HFN2-15, and HFN2-30, depending on the initial silver salt concentrations and stirring times. The morphology of the HH and size of AgNPs were examined using Environmental Scanning Electron Microscopy-ESEM-EDAX (ESEM FEI Quanta 200) and the elemental content of AgNPs was confirmed from Energy dispersive X-ray (EDX) spectroscopy, using an accelerating voltage of 15 kV and an emission current of 12 μA. The basic information regarding functional groups presented in the HH that helped the bio reduction of silver ions and capping of AgNPs was determined using Fourier transform infra-red (FTIR) spectrophotometer (FT-IR Spectrometer Spectrum Two, PerkinElmer). The spectra of each sample were collected at a resolution of 4 cm^{-1} and 16 interferogram scans in the range of 400 - 4,000 cm^{-1}. All observed absorption peaks were assigned to the best of our knowledge to confirm the various functional groups that were present in the hemp hurd (HH) and AgNPs solutions.

2.5. Antibacterial Activity of Silver Nanoparticles (AgNPs)

Both Gram-negative (*E. coli* 25,922) and Gram-positive (*S. aureus* 25,923) pathogenic waterborne bacteria were tested to investigate the antibacterial activity of so formed HHC stabilized AgNPs. An amount of 30.5 g of the Mueller-Hinton agar, dehydrated culture media was dissolved in 1L of deionized water and then heated to dissolve completely. Disk diffusion tests were conducted to determine bacterial sensitivity towards AgNPs [26]. The Luria agar was sterilized in the autoclave at 120˚C for 15 minutes. After cooling to around 45˚C, the broth was dispensed into sterile Petri dishes. A 3-way swab plate was prepared on an agar plate and filter disks (~5 mm diameter) were placed at intervals on the surface of plates using sterile forceps. The plates were incubated at 37˚C for 24 hours. 10 μl aliquots of 1 mM and 5 mM AgNPs were impregnated on filter disk having uniform bacterial suspensions of *E. coli* and *S. aureus*. The diameter of the zone of inhibition (ZoI) was measured using photographic images of the agar plates [27].

Using the broth dilution method, the Minimum Inhibitory Concentration (MIC) of AgNPs was determined and the bactericidal effect of AgNPs was evaluated based on the Minimum Bactericidal Concentration (MBC). The MIC was defined as the lowest concentration of AgNPs, which inhibited bacterial growth. The MBC value was defined as the lowest concentration of nanoparticles that prevented the visible growth of bacteria on the agar plates after incubation. A

volume of 0.2 mL of each bacterial strain was added to the test tube containing 1 ml broth (cell numbers reach ~10^8 - 10^9 CFU/mL after overnight grown culture) and was mixed with a serial dilution of 1 mM (HFN1) AgNPs. Positive controls contained 1 mL of Luria broth and 0.2 mL of *E. coli* and *S. aureus* strain without containing AgNPs. The negative control contained only Luria broth. Tubes were incubated at 37˚C for 24 hours. Each sample was examined for bacterial growth by monitoring the changes in the visual turbidity with the naked eye. The tubes that appeared to have no or little growth were plated on Luria broth agar plates to differentiate between bactericidal and/or bacteriostatic effect and these plates were incubated at 37˚C for another 24 hours.

3. Results and Discussion

The formation of the AgNPs in the solution was always accompanied with a distinct color change arising from SPR of AgNPs in solution as noted in **Figure 1**. The concentrated HHC that exhibited a pale-yellow color (Sample **A**), turned colorless on dilution with silver salt solution (Sample **A′**). The colorless sample **A′** on heating resulted in two different degrees of brown colors, depending on the heating time of the sample. At the fixed concentration of HHC and silver salt, the colorless solution initially turned into pale brown color on heating for 15 minutes and then on continuously heating for 30 minutes for the same solution turned into a dark-brown color. This color change was an indication of the successful formation of AgNPs, which occurred in the hemp hurd extract due to the reduction of Ag^+ ions to metallic Ag^0 in the nanoscale, akin to our previous work [5]. The UV-Vis absorption spectra of the samples were recorded at 15 and 30 minutes of heating of HHC containing 1 mM and 5mM silver nitrate precursor. The solution of HHC containing AgNPs exhibited UV-Vis absorption spectra with a peak maximum of around 450 nm originating from surface plasmon resonance (SPR) of AgNPs (**Figure 2**), well-documented in the literature [28]. **Figure 2** showed the UV-Vis spectra of the AgNPs stabilized within hemp hurd extract at two different concentrations (HFN1-1 mM) and (HFN2-5 mM) of $AgNO_3$ and at two different stirring times. The spectra were in good agreement with the reported literature [5] [28] indicating the formation of spherical AgNPs within the HHC solution. The broad UV-Vis spectra indicated the presence of different sized AgNPs in solution. As expected, the ESEM images in **Figure 3(a)** revealed the filamentous and porous structure of the fiber (HH). There were some interfibrillar gaps that were detected on the surface of the untreated hemp hurd. The ESEM image (**Figure 3(b)**) showed the formation of relatively uniform size silver nanoparticles from hemp hurd extract. **Figure 4** showed the particles were indeed spherical and exhibited a size range of 200 - 300 nm. Both the images (**Figure 3(b)** and **Figure 4**) clearly showed some aggregation of particles within the hemp matrix; the aggregation could be due to the concentration effect in the solid form when the sample was dried. The SEM images showed that most of the AgNPs were spherical or quasi-spherical in shape as expected from UV-Vis

data. In addition to simple ESEM images, the elemental characterization of so formed AgNPs within the HHC media was also performed using EDX spectroscopy. The major peaks appeared around 3 keV confirming the chemical identity of nanoparticles stabilized within the HHC extract. These results demonstrated the first-ever formation of AgNPs within HHC media upon heating by facile "green synthesis" method. The size and shape tuning need to be investigated further.

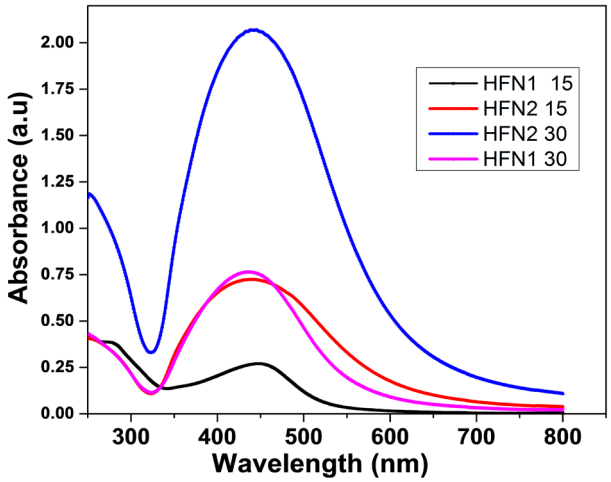

Figure 2. UV-Vis absorption spectra of AgNPs synthesized from HHC at (a) HFN1-1 mM $AgNO_3$ concentrations, 15 minutes of heating (b) HFN2-5 mM $AgNO_3$ concentrations, 30 of minutes heating.

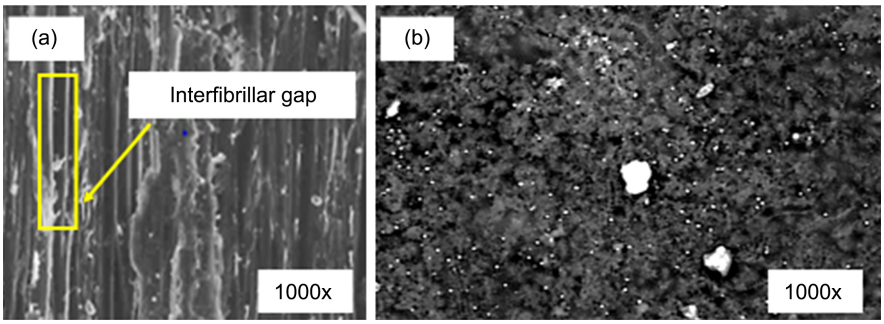

Figure 3. ESEM images of (a) dried HH and (b) HHC stabilized AgNPs.

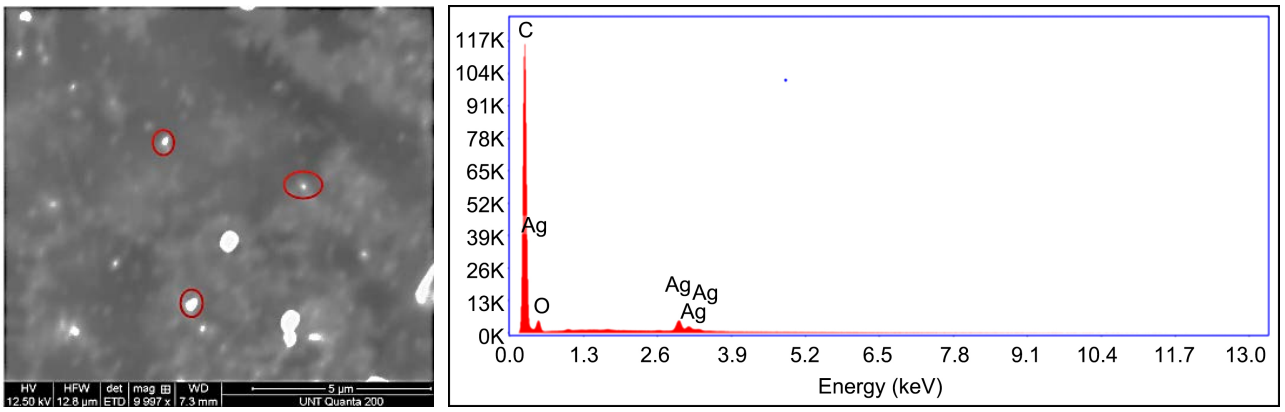

Figure 4. EDX image (left) and EDX spectra (right) of HHC stabilized AgNPs.

The FTIR data obtained for the HHC and AgNPs stabilized within the HHC are shown in **Figure 5**. The spectra indicated the stretching and bending vibrations of diverse functional groups within the samples. Three strong peaks appeared at 599, 1,032, and 3,336 cm^{-1} for hemp hurd extract. These absorption peaks represented the C-H vibration from cellulose, C-C stretching vibration or C-OH side group vibration, and -OH stretching respectively [29]. Four strong peaks were noticed in the spectra of hemp-stabilized silver nanoparticles sample at 792, 1,310, 1,628, and 3,401 cm^{-1}. Peaks at 3,401 cm^{-1} represent OH-stretching, showing the presence of phenols. The stretching vibrations at 1,628 cm^{-1} are attributed to the C=O stretch of the amide bonds. Characteristic absorption at 1,310 cm^{-1} represents the C-N stretching due to the presence of aromatic amino group, while 792 cm^{-1} represented the bending vibration of the C-H of the aromatic lignin group. These general assignments of IR bands were based on the literature [30] [31]. The HHC media being extracted from the plant source is expected to exhibit these chemical identities. These different chemical identities could play a crucial role in reducing the Ag(I) species to Ag(0) as well as for stabilizing the AgNPs [32].

The antibacterial activity of the AgNPs was investigated against both the Gram-positive and Gram-negative bacteria using the agar well diffusion assay. **Figure 6** showed the zones of inhibition (ZoI) of the two strains of bacteria. The ZoI for *Staphylococcus aureus* and *Escherichia coli* bacteria for HHC, HFN1, and HFN2 samples were found to be around 15, 16, and 19 mm, respectively. It was noticed from **Figure 6** that the ZoI was significantly smaller for HHC vs HHC-stabilized AgNPs as expected though pharmacological properties of HHC were reported [32], no antibacterial activity was detected from the HHC control solution in the present work.

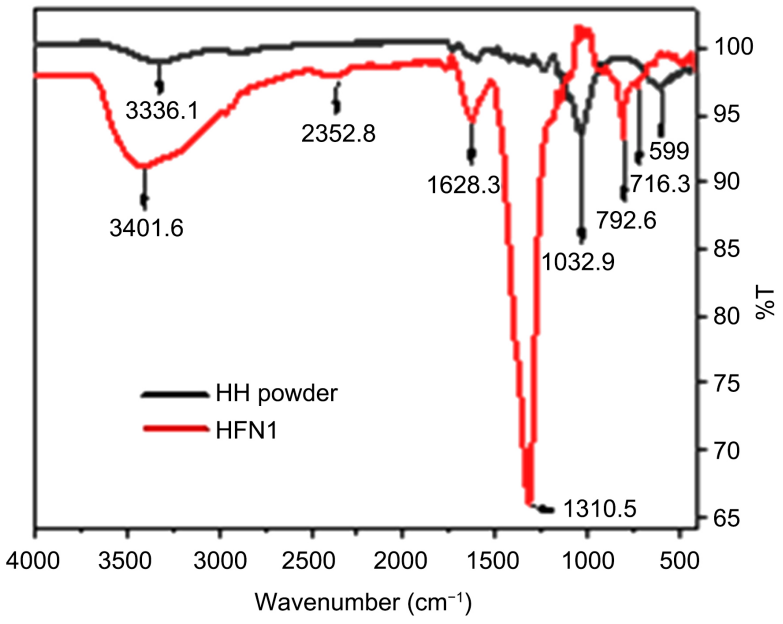

Figure 5. FT-IR spectra of dried HH and HHC synthesized AgNPs.

Table 1. Antibacterial activities of silver nanoparticles prepared from various plant extract.

		Zone of inhibition (mm)			
Plants related to HEMP species or similar plants	Mode of synthesis	Size of AgNPs	*E. coli*	*S. aureus*	References
Kenaf (*Hibiscus cannabinus*) leaves	Prolonged heating	~100 nm	11	No activity	[33]
Jute fiber	Acid treatment	500 - 600 nm	18	15	[34]
Hemp hurd (*Cannabis sativa*)	Heated at 70˚C for 30 mins	100 - 200 nm	18	16	Our work

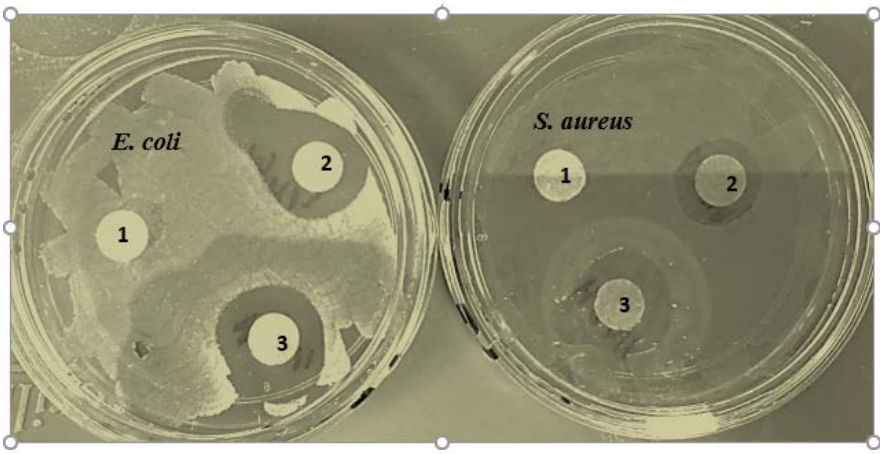

Figure 6. Antibacterial activity of (1) HHC, (2) HFN1, and (3) HFN2.

The bacteriostatic and bactericidal effects of synthesized nanoparticles have been screened by the determination of MIC and MBC against *E. coli and S. aureus*. In the primary screening, AgNPs showed a strong antibacterial activity with the MIC concentration of 12.77 μg/mL and the MBC concentration of 25 μg/mL against both the pathogens respectively.

In order to understand the antipathogenic effects of HHC stabilized AgNPs, the results from our work were compared with various plant extract stabilized AgNPs from the literature. Table 1 showed the antibacterial activity of selective and most popular plant extract stabilized AgNPs in comparison with the present work.

4. Conclusions

A green synthesis method for silver nanoparticles using dry hemp hurd extract was developed and demonstrated. The synthesis route is simple, single-step, low energy-based, and benign, eliminating the usage of any hazardous chemicals to deem it "green". The formation of silver nanoparticles was confirmed from visual color change, UV-Vis, ESEM, and EDX data. The elemental analysis of AgNPs confirmed the presence of elemental silver with negligible traces of carbon and oxygen as expected. The synthesized AgNPs showed strong potential against both Gram-positive and Gram-negative bacteria. The antibacterial activity of the AgNPs against the Gram-positive *S. aureus* and Gram-negative *E. coli* was al-

most identical. Nevertheless, this emerging innovation could become a substitute for chemically synthesized AgNPs and significantly improve the application of hemp hurd extract towards water filtration applications.

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Conflicts of Interest

The authors declare there is no conflict of interest regarding the publication of this research manuscript.

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