

Biogenic Synthesis and Characterization of Silver Nanoparticles Using *Hoya Carnosa* Flower Extract

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ABSTRACT

Silver nanoparticles (AgNPs) are important metallic nanomaterials thanks to their superior antibacterial properties and wide application areas; however, conventional synthesis methods are quite limiting in terms of environmental and economic aspects. This situation causes green synthesis methods to be investigated more and more. *Hoya carnosa* plant have the potential to be an alternative biogenic synthesis agent to traditional methods thanks to their rich phytochemical content. In this study, biogenic AgNPs were synthesized for the first time using *Hoya carnosa* flower extract. The reaction was completed in a short time, such as 15 min under room conditions. AgNPs were comprehensively characterized by UV-Vis, FTIR, XRD, SEM, TEM and EDX analyses. According to the obtained results, the average size of AgNPs was calculated as 16 nm by the Debye Scherrer equation and 21 nm by TEM analysis. This study demonstrates that the use of *Hoya carnosa* flower extract can be a successful alternative to traditional methods by contributing to the synthesis of AgNPs in an environmentally friendly and rapid manner.

Keywords: Green synthesis, Biogenic synthesis, Silver nanoparticles, *Hoya carnosa*, Plant.

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Introduction

Nanotechnology attracts attention as one of the most important fields that has been frequently mentioned in recent years, and research on the development and use of nanosized materials is gaining momentum day by day. In this context, metal nanoparticles serve numerous purposes in different areas—from environmental [1] and biomedical [2] fields to electronics [3], energy [4], food [5], agriculture [6], textile [7], and cosmetics [8]—thanks to their unique optical, magnetic, electrical, biological, and chemical properties [9,10]. Especially silver nanoparticles (AgNPs) have attracted great attention in healthcare and many other industries due to their antimicrobial, anti-inflammatory, and anticarcinogenic impacts [11,12]. However, conventional chemical and physical AgNPs synthesis techniques bring with them potential dangers in terms of the use of toxic chemicals, high energy consumption, and environmental sustainability [13,14].

In recent years, biogenic synthesis methods have attracted considerable attention as an eco-friendly and sustainable alternative to reduce the negative effects of conventional methods. Biogenic synthesis is based on the utilization of bio-based reducing agents such as plant extracts, bacteria, yeast, fungi, and algae and offers safe and sustainable solutions in terms of health, environment, energy, and economy [15,16]. Plant extracts, especially due to their rich phytochemical content such as phenolic compounds, flavonoids, terpenoids, alkaloids, and saponins, effectively reduce silver ions and ensure the formation of stable AgNPs [17]. The function of these phytochemicals as reducing and stabilizing agents provides the opportunity to control the size, shape, and surface properties of nanoparticles.

Hoya carnosa is a plant belonging to the Asclepiadaceae family and is generally grown for ornamental purposes, and is used in traditional medicine for various therapeutic purposes due to its antioxidant, antimicrobial, and anti-inflammatory effects [18–22]. The flowers have a pentamerous corolla with a prominent star-shaped gynostegium formed by fused male and female organs at the center (Figure 1) [23]. Extracts obtained from its leaves and flowers serve as potential therapeutic agents in biomedical applications due to their rich phytochemical content [21]. However, current studies have generally focused on the biological processes and pharmacological properties of *Hoya carnosa*, and no study has yet been found that has investigated its potential use in nanoparticle synthesis.



Figure 1. *Hoya carnosa* flowers.

In this study, for the first time in the literature, AgNPs were synthesized by biogenic methods using *Hoya carnosa* flower extract. This method is quite simple and provides biocompatible production of nanoparticles in accordance with green chemistry principles without using any toxic chemicals. In addition, the chemical, morphological, and structural properties of the synthesized AgNPs were characterized by detailed examination with advanced spectroscopic and microscopic approaches, including UV-Vis, FTIR, XRD, SEM, TEM, and EDX.

Materials and Methods

Materials

Hoya carnosa flowers were freshly collected in May from a mature potted plant grown in a local home in Sivas, Türkiye. Silver nitrate (AgNO_3) was obtained from Carlo Erba Reagents (Italy). MilliQ ultrapure water (Millipore Synergy UV, Merck, Germany) was used in all stages of the experiments.

Preparation of Flower Extract

Hoya carnosa flowers were freshly collected and washed first with water and then with ultrapure water to remove dirt and dust. After the flowers were separated from their stems, they were chopped into small pieces. 20 g of fresh *Hoya carnosa* flowers were extracted by boiling in 250 ml of ultrapure water for 30 min. The resulting pink extraction solution was allowed to cool and filtered first with a simple strainer and then with Whatman No. 1 filter paper. The extract obtained was stored in the refrigerator at 4 °C.

Biogenic Synthesis of AgNPs

10 ml of *Hoya carnosa* flower extract was taken into a skirted centrifuge tube. Instead of preparing a separate silver solution, 1 mmol of AgNO_3 was directly added to the extract solution and mixed briefly with a vortex mixer until all solids were dissolved. After shaking the tube at 100 rpm for 15 min at room temperature in an orbital shaker, a dark colloidal solution was formed, indicating that the reduction reaction had taken place and successfully ensured the nucleation and growth of spherical AgNPs, as reported in the literature (Figure 2) [24]. The synthesis mechanism begins with coordination of Ag^+ ions by phenolic –OH groups, followed by electron donation that reduces Ag^+ to Ag^0 , nucleation and growth of silver seeds, and final stabilization of the nanoparticles through capping by oxidized phytochemicals [25]. The AgNPs solution was centrifuged at 6000 rpm for 10 min to precipitate the particles. This procedure was repeated three times by adding ultrapure water instead of the drained supernatant, and finally pure AgNPs were obtained. The obtained nanoparticles were dried at 50 °C. The yield of synthesized AgNPs was determined using Equation 1.

$$\% \text{ Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100 \quad (1)$$

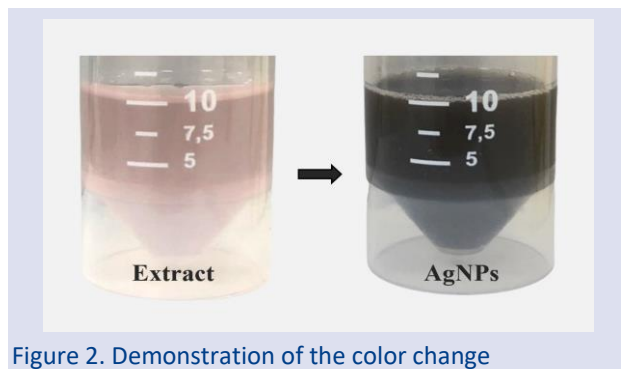


Figure 2. Demonstration of the color change

Characterization of AgNPs

The characterizations of AgNPs synthesized by biogenic methods using *Hoya carnosa* flower extract were carried out by UV-Vis, FTIR, XRD, SEM, EDX and TEM techniques. The presence of AgNPs was confirmed by UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, USA) and the chemical structure analysis was determined by FTIR-ATR (Tensor II ATR, Bruker, USA). XRD (Mini Flex 600, Rigaku, Japan) was used for the determination of crystal structure and theoretical particle size calculations. The size, morphology and elemental analyses of nanoparticles were characterized using SEM (Mira 3, Tescan, Czech Republic) integrated with S-TEM and EDX (X-act, Oxford Instruments, UK) detectors.

Results

UV-Vis Analysis

UV-Vis absorption spectroscopy is a highly effective method for the detection of the presence of many metallic nanoparticles, especially silver and gold nanoparticles, using Surface Plasmon Resonance (SPR) peaks [26]. The presence of AgNPs synthesized by biogenic method using *Hoya carnosa* flower extract was analyzed by observing the SPR peak using UV-Visible spectrophotometer in the wavelength range of 350 to 800 nm and shown in Figure 3. The analysis was performed in a quartz cuvette with a 1 cm light path and at 25 °C. The λ_{max} value at 436 nm wavelength resulting from the SPR effect confirms the successful formation of AgNPs in the extract solution. In previous studies in the literature, the maximum wavelength of AgNPs has generally been reported in the range of 400–450 nm [27].

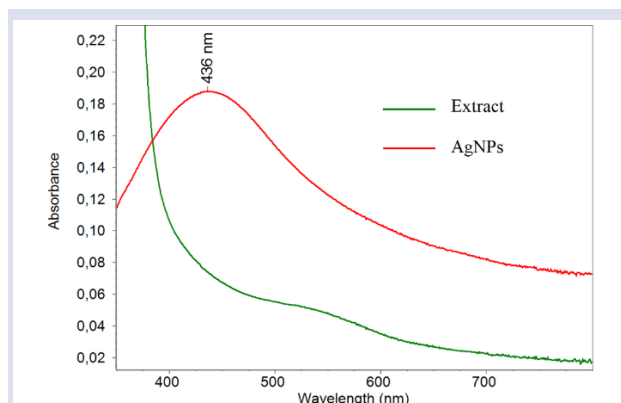


Figure 3. UV-visible spectra of *Hoya carnosa* Extract and AgNPs.

FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is one of the most fundamental techniques used to investigate the presence of functional groups in metallic nanoparticles. The synthesized AgNPs were characterized with 16 scan numbers in the wavenumber range of 4000-400 cm^{-1} and the FTIR-ATR spectrum is presented in Figure 4. The strong band at 3450 cm^{-1} is attributed to the O-H stretching vibrations of phenols and the N-H stretching vibrations of proteins [28, 29]. The medium sharp band centered at 1633 cm^{-1} is assigned to the N-H bending vibrations originating from the amines in the protein structure [28]. The weak band at 1384 cm^{-1} is attributed to the O-H bending vibrations of phenols [30]. The synthesis of the nanoparticles occurred via a reduction reaction, especially with the phenolic compounds in the plant extract acting as both reducing and stabilizing agents and thus constituting the main phytochemical group responsible for this process [31]. The broad bands at 1112 cm^{-1} and 559 cm^{-1} may be due to the C-N stretching vibrations of the amine groups and halo compounds, respectively.

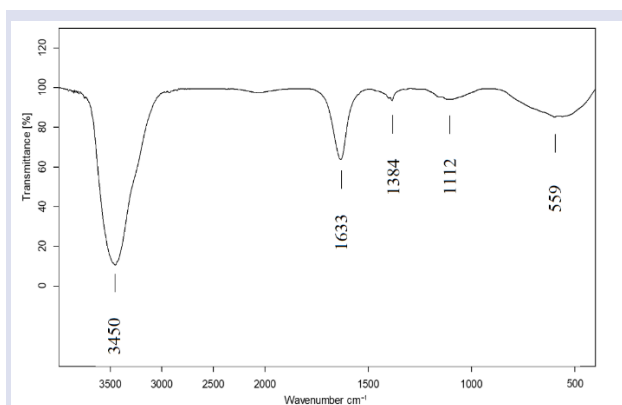


Figure 4. FTIR spectra of the biogenic synthesized AgNPs.

XRD Analysis

XRD analysis was carried out at a voltage of 40 kV and a current of 15 mA with Cu K α radiation in the region of 2 θ from 20° to 80° and the spectrum of AgNPs are shown in Figure 5. The diffraction peaks at 2 θ values of 37.90°, 44.13°, 64.36°, and 77.38° are attributed to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes, respectively. X-ray diffraction (XRD) patterns of silver nanoparticles show that its structure is face-centered cubic and match well with standard patterns of silver (JCPDS file no. 04-0783) [32, 33]. Since the analysis was performed with a low amount of AgNPs, the remaining peaks belong to the silicon originating from the sample holder. The average crystalline size of the AgNPs was calculated using the Debye Scherrer equation shown in Equation 2.

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (2)$$

Here, D is the crystalline size of the nanoparticles, K is the Debye Scherrer constant (0.98), λ is the wavelength of Cu K α radiation (0.154 nm), and β is the full width at half

maximum (FWHM) [34]. The theoretical average crystalline size (D) of the biogenic synthesized AgNPs was determined as 16 nm according to the Debye-Scherrer equation.

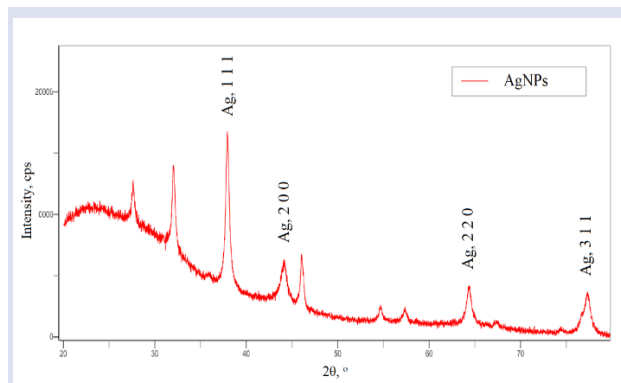


Figure 5. Powder XRD pattern of the biogenic synthesized AgNPs.

SEM, TEM and EDX Analyses

Scanning electron microscopy (SEM) is undoubtedly the most widely used technique for characterizing the shape, size and surface morphology of nano-sized materials. AgNPs, synthesized using *Hoya carnosa* flower extract using a very simple, fast, economical and environmentally friendly method, were examined under 100 kx magnification using a secondary electron (SE) detector at 20 kV and shown in Figure 6.a. It is clearly seen that the nanoparticles, which are highly agglomerated due to drying process, are mostly spherical in shape and their sizes are below 100 nm.

Transmission electron microscopy (TEM) is another powerful technique that provides much higher resolution than other techniques due to the high voltage values it uses. Since the nanoparticles are dispersed in the liquid, agglomeration is largely eliminated and thus the size distribution and morphological properties of the nanoparticles can be measured directly. In this study, TEM analyses were examined under 250 kx magnification at 30 kV with an S-TEM module connected to the SEM device and the resulting image is presented in Figure 6.b. It was also confirmed by TEM that AgNPs were mostly spherical in shape and had a size below 100 nm. The diameters of 90 individual nanoparticles were measured from the SEM image (Figure 6.a) using the Mira TC software, and the average particle size was determined to be 21 nm. In addition, this value is very close to the theoretically calculated size of 16 nm from the XRD peaks.

Energy-dispersive X-ray spectroscopy (EDX) is a useful analysis technique for elemental and compositional characterization of the materials using the characteristic X-rays of electrons. EDX spectrum of AgNPs is given in Figure 6.c. The results show that approximately 93% of the nanoparticles by weight consist of silver element. The carbon and oxygen content in the material are thought to originate from phytochemicals bound to silver ions and residues of uncleaned *Hoya carnosa* flower extract. Similarly, the phytochemical content is also found in the FTIR spectrum, indicating that the results are consistent.

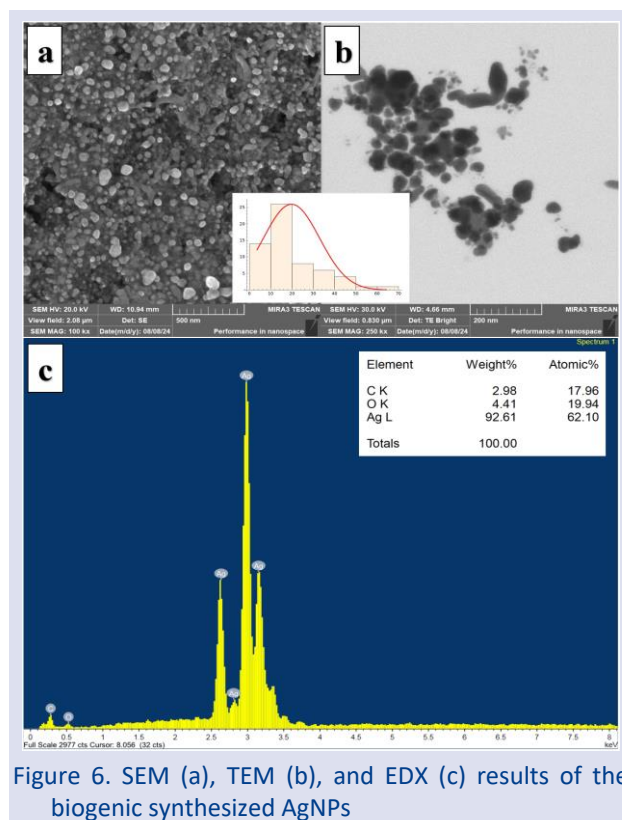


Figure 6. SEM (a), TEM (b), and EDX (c) results of the biogenic synthesized AgNPs

The synthesis of silver nanoparticles from flower extracts, like other parts of plants including leaves, stems, and roots, has recently become a widely researched topic. In this context, a comparative list of studies conducted in the literature on AgNP synthesis using flower extracts is presented in Table 1. When compared to other studies, it is clearly seen that AgNP synthesis from *Hoya carnosa* flower extract is a successful method with its very short reaction time and the possibility of synthesis under room conditions. In addition, the nanoparticle size measured at 21 nm is at a level that is competitive with AgNPs produced from other flower extracts reported in the literature.

Table 1. Recent studies on AgNP synthesis using flower extracts.

	Flower used for extract	Reaction duration	Reaction temperature	AgNPs size (nm)	Reference
1.	<i>Ferulago macrocarpa</i>	2.5 h	80 °C	14-25	[35]
2.	<i>Plumeria alba</i>	30 min	Room temp.	36.19	[36]
3.	<i>Caesalpinia pulcherrima</i>	24 h	Room temp.	12	[37]
4.	<i>Fritillaria</i>	2 h	Room temp.	10	[38]
5.	<i>Moringa oleifera</i>	30 min	-	8	[39]
6.	<i>Ipomoea digitata</i>	10 min	80 °C	<100	[40]
7.	<i>Madhuca longifolia</i>	20 min	40 °C	30-50	[41]
8.	<i>Tussilago farfara</i>	4 h	80 °C	13.57 ±3.26	[42]
9.	<i>Malva sylvestris</i>	4 h	25 °C	20-40 nm	[43]
10.	<i>Hoya carnosa</i>	15 min	Room temp.	21 nm	This study

Conclusion

In this study, AgNPs were synthesized via a biogenic method using *Hoya carnosa* flower extract, which represents a low-cost, environmentally sustainable, and non-toxic green synthesis approach. The synthesis was completed under room conditions within a short time of 15 min, achieving a 64 % yield. The AgNPs were analyzed using UV-Vis, FTIR, XRD, SEM, TEM, and EDX analyses. The successful synthesis of the AgNPs was confirmed by the SPR peak at 436 nm in the UV-Vis analysis, while FTIR verified the presence of phytochemical components attached to the nanoparticles. XRD analysis revealed the face-centered cubic crystal structure of the AgNPs, and the size was calculated as 16 nm using the Debye Scherrer equation. SEM and TEM analyses demonstrated that the nanoparticles were spherical, with a particle size of 21 nm. EDX results further confirmed the presence of silver and phytochemical content. This study is the first report of the biogenic synthesis of AgNPs from *Hoya carnosa* flower extract, demonstrating that this plant can serve as a successful alternative to conventional AgNPs synthesis methods. Future studies may explore the antibacterial, antioxidant, and antifungal properties of these biogenic AgNPs, potentially expanding the use of *Hoya carnosa* in scientific research.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgments

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