RESEARCH ARTICLE

Green synthesis of silver nanoparticles using Verbena officinalis extract and investigation of their structure, size and stability

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ABSTRACT

In this study, silver nanoparticles (AgNPs) were green-synthesized using Verbena officinalis extract and various analyses were performed to prove the formation of silver nanoparticles. UV–Vis spectroscopy showed successful synthesis of silver nanoparticles with an absorption peak at 384 nm and FT-IR analyses also confirmed the role of active plant compounds, such as polyphenols and proteins, in reducing silver ions and stabilizing the nanoparticles. X-ray Diffraction (XRD) analysis indicated a face-centered cubic crystalline structure with a crystallite size of 22.67 nm. Scanning Electron Microscopy (SEM) images further validated the spherical morphology and uniform size distribution of the nanoparticles. Additionally, zeta potential and polydispersity index (PDI) measurements confirmed the high stability of the synthesized nanoparticles that attributed to the reducing and stabilizing agents in extract. This study highlights the efficiency of V. officinalis extract in synthesizing small, stable silver nanoparticles. The proposed method offers an environmentally friendly and effective approach for nanotechnology applications, with potential uses in biomedical and environmental industries.

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INTRODUCTION

Nanotechnology is among the most dynamic and rapidly evolving fields in contemporary material science. The synthesis and study of noble metal nanoparticles, such as silver, gold, and platinum, have garnered significant attention due to their versatile applications. These nanoparticles play a crucial role in areas such as biotechnology, as well as electronics,

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optoelectronics, magnetics, biomedical technologies, and information storage systems [1-3]. Among various nanomaterials, silver nanoparticles (AgNPs) are particularly prominent. Renowned for their exceptional antimicrobial properties, AgNPs are widely utilized in a variety of products, including personal hygiene items, cleaning and disinfecting agents, construction materials, medical devices, textiles, and even in food processing industries [4].

In the 21st century, Nanobiotechnology has emerged as one of the most promising fields of science due to its attractive mechanisms and wide-ranging applications in biotechnology, chemistry, medicine, and materials science [5,6]. One of the most notable examples of these applications is the use of metals in nanoparticle preparation [7]. Among various types of nanomaterials, silver nanoparticles (AgNPs) have drawn considerable attention due to their unique properties and diverse advantages in areas such as electronics, optics, catalysis, and particularly antimicrobial uses [8-11]. AgNPs have demonstrated potent antioxidant, antibiofilm, anticancer, antifungal, anti-inflammatory, antiviral, anti-angiogenesis, and larvicidal properties [2,12,13].

There are several methods available for the synthesis of metal nanoparticles, including chemical, electrochemical, photochemical, and radiation-based approaches [14]. Each method has its own set of advantages and disadvantages, with common challenges such as high costs, scalability, uniform particle size, and distribution. Among these methods, chemical approaches have been most commonly employed for the production of AgNPs. The chemical reduction of metal ions remained as one of the most straightforward and widely used methods for the preparation of metal nanoparticles [15]. A variety of chemical techniques have been developed for synthesizing silver nanoparticles, including chemical reduction, aqueous chemical reduction, nonaqueous chemical reduction, the template method, electrochemical reduction, ultrasonic-assisted reduction, photo-induced or photocatalytic reduction, microwave-assisted synthesis, irradiation reduction, and the micro-emulsion method [16].

The use of hazardous substances is often unavoidable in many nanoparticle synthesis methods, leading to risks such as chemical precursor contamination, solvent toxicity, and the generation of harmful byproducts [17]. These concerns highlight the need for safer, more sustainable alternatives. One promising solution lies in green chemistry, which leverages the complex biological pathways and resources of living systems—such as bacteria, fungi, algae, viruses, plants, and plant extracts—for the biosynthesis of nanoparticles [18].

This biosynthesis method is simple, requiring less time and energy compared to traditional physical and chemical methods, with more predictable mechanisms. Other advantages of biological methods include the wide availability of biological resources, reduced time requirements, high particle density, enhanced stability, and the ease with which nanoparticles can be dissolved in water [19]. Natural reducing agents, such as plant extracts, cow milk, and the extracellular products of bacteria and fungi, are frequently utilized in the production of AgNPs [20].

Plant extracts play a crucial role in reducing Ag^{+} ions into nano crystallites. They are particularly advantageous due to their ease of use, lack of complex culture maintenance, and potential for large-scale production [21]. The biogenic molecules present in these extracts help in the self-assembly and capping of metal nanoparticles, which can also assist in controlling their shape during the reduction process [22]. Additionally, many components of these plant extracts exhibit antimicrobial activity against both animal and plant pathogens. Numerous studies have reported the successful biosynthesis of AgNPs using plant species that have shown potential in the biosynthesis of nanoparticles [23,24]. As the

antimicrobial properties of medicinal plants gain recognition, research is increasingly focused on establishing the antibacterial effects of plant extracts used in nanoparticle synthesis, as well as the combined effects of the plant extract and metal nanoparticles [25]. This approach opens up opportunities for developing nanoparticles as effective antibacterial agents, particularly for combating emerging multi-drug-resistant pathogens. Furthermore, the synergistic combination of biocompatible medicinal plants with nanoparticles holds promise for new therapeutic applications, particularly in treating infections caused by organisms that have developed resistance to conventional antibiotics [26].

Verbena officinalis L. (Verbenaceae), commonly known as Atuch, is a weed native to semi-arid regions in Ethiopia. This plant is well-regarded in folk medicine for its anti-inflammatory, diuretic, and expectorant properties, as well as its antibacterial effects [27]. It has long been used in traditional medicine to treat various ailments, including skin burns, abrasions, and gastrointestinal disorders [28,29].

In this article, the green synthesis of silver nanoparticles using Verbena officinalis extract has been studied for use as a suitable and available alternative for the synthesis of silver nanoparticles.

EXPERIMENTAL

Preparation of Verbena officinalis Extract

Absolute ethanol was poured as the solvent in the flask of the Soxhlet extractor. Then, the herb was weighed and placed in the siphon arm. The heat was set to 78°C, and the alcohol began vaporization and flowed into the siphon. This process occurred several times to make the green liquid in the siphon colorless, suggesting the full separation of herbal components. Then, the rotary evaporator was used to separate the herbal extract. Once the solvent had been fully separated, a concentrated verbena officinalis extract remained.

Green Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized using a green biosynthesis method based on the protocols of Asadi et al [2], with slight modifications. Extracts of Verbena were employed as the reducing agent. To initiate the synthesis, 5 mL of the extract was added to 95 mL of 1 mM aqueous silver nitrate ($AgNO_3$) solution in a 250 mL conical flask. The mixture was incubated at room temperature on a shaker for 24 hours. The bio-reduction of $AgNO_3$ to AgNPs was visually confirmed by the development of a dark brown color in the solution.

Following the incubation period, the synthesized AgNPs were harvested by centrifugation at 12,000 rpm for 15 minutes. The resulting pellets were washed twice with distilled water to remove impurities. Subsequently, the purified AgNPs were calcined at 250 °C for 1 hour. The calcined products were then washed once with double-distilled water to obtain the final nanoparticle samples.

Characterization of Green Synthesized AgNPs

UV-Vis Spectrophotometry

The bio-reduction of Ag^+ ions into silver nanoparticles was monitored using a UV-Vis spectrophotometer (SP65 Spectrophotometer). The UV-Vis spectra of the reaction medium were recorded at room temperature, using a double-beam scanning spectrophotometer in the wavelength range of 300–700 nm. A 1 mM silver nitrate solution served as the blank control.

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared Spectroscopy (FT-IR) was conducted to identify the functional groups present in the synthesized AgNPs. The spectra were recorded using an FT-IR spectrophotometer (Serial# C109832) with a resolution of 2 cm^{-1}, covering the range of 4000–400 cm^{-1}.

X-Ray Diffraction (XRD)

The crystalline structure of the green-synthesized AgNPs was analyzed using X-Ray Diffraction (XRD) with a Shimadzu X-ray Diffractometer (PXRD-700). The diffraction patterns were recorded in the 2Θ range of 30–80° using CuKα radiation with an energy of 8.04 keV and a wavelength of 1.54 Å. The system was operated at 40 kV and 25 mA. The average crystalline size of the nanoparticles was calculated from the XRD pattern using the Scherrer equation, based on the line width of the maximum intensity reflection peak.

Scanning Electron Microscopy (SEM)

The morphology of AgNPs was studied using Scanning Electron Microscopy (SEM) (KYKY, Model No. EM 3200). The nanoparticles were centrifuged at 10,000 rpm for 15 minutes, and the supernatant was collected. The nanoparticle pellet was deposited onto glass slides and air-dried at room temperature before imaging.

Dynamic Light Scattering (DLS) and Zeta Potential Analysis

The size distribution, polydispersity index (PDI), and zeta potential of the synthesized AgNPs were measured using a Nanoplus-3 particle/zeta analyzer (Micromeritics, Norcross, GA, USA). The nanoparticle suspension was sonicated and diluted with double-distilled water prior to analysis to ensure uniform dispersion.

RESULTS AND DISCUSSIONS

Visual observation

During the synthesis, first the mixture appeared milky white, but within an hour, it changed to brown in the test tubes containing both the silver nitrate solution and plant extracts. This color change signifies the formation of silver nanoparticles (AgNPs). Conversely, no color change was observed in the control tube containing only silver nitrate, confirming that the plant extract played a critical role in nanoparticle synthesis.

After 24 hours of incubation at room temperature the color of the mixture intensified to a deep brown, confirming an exponential increase in the formation of AgNPs as the reaction progressed. Similar observations have been reported in previous studies. For instance, Dongyang Wang et al. (2021) noted a transition from yellow to brown during silver nanoparticle synthesis, while Kiran et al. (2021) observed a shift from faint reddish to dark brown [32,33]. These changes corroborate the time-dependent synthesis and accumulation of AgNPs in the reaction mixtures.

Characterization by UV-Vis Spectrophotometer

The formation of silver nanoparticles (AgNPs) through the reduction of silver ions by V. officinalis extracts was confirmed using UV–Vis spectroscopy, a widely recognized technique for monitoring nanoparticle synthesis and stability [34]. As shown in Figure 1, the absorption spectrum of synthesized AgNPs using the aqueous extract of V. officinalis at room temperature after 24 hours of incubation revealed a surface plasmon resonance (SPR) peak at 384 nm.

UV–Vis spectroscopy is crucial for validating the synthesis of AgNPs, as their characteristic dark brown color arises from the excitation of SPR. This phenomenon occurs due to the interaction of light with the free electrons on the surface of silver nanoparticles [2]. The typical SPR absorption band for AgNPs is reported within the range of 400–500 nm, but the observed peak at 384 nm falls slightly below this range. This deviation might result from the absorption of silver ions, complexes, impurities, or phytochemicals present in the plant extract [35].

The SPR characteristics are influenced by nanoparticle shape, size, and the dielectric constant of the surrounding medium [36]. For instance, the study by Providence et al. (2020) demonstrated absorption

maxima ranging from 285 to 350 nm during the synthesis of AgNPs using Kigelia africana extract [35]. The shift to longer wavelengths with reduced intensity over time indicated an increase in particle size. Similarly, broad peaks at higher wavelengths signify larger particle sizes, while narrow peaks at shorter wavelengths are associated with smaller nanoparticles [37].

In this study, the observed UV–Vis spectra suggest successful conversion of silver ions to AgNPs, with a stable SPR peak indicating nanoparticle formation. The presence of a secondary peak might result from the aggregation of some nanoparticles. Literature also supports the role of plant extract concentration in affecting nanoparticle size and size distribution [38].

Characterization by Fourier Transform-Infrared (FT-IR) Spectroscopy

Fourier Transform-Infrared (FT-IR) spectroscopy was employed to identify the functional groups present in the synthesized silver nanoparticles (AgNPs) and to evaluate the reducing and stabilizing roles of V. officinalis extracts. The analysis was conducted in the wavelength range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} using KBr pellets.

The FT-IR spectra revealed several characteristic absorption bands, indicating the presence of various functional groups (figure 2). The absorption bands at 2735.12 cm^{-1} correspond to the stretching or bending vibrations of C–H alkenes. Additionally, the bands observed at 3391.26 cm^{-1} is attributed to the stretching and bending vibrations of carboxylic acids.

Protein-associated vibrations were identified around 1191.93 cm^{-1}, which may be assigned to amide I and II N–H bending due to carbonyl stretching and peptide linkages of proteins, as supported by previous studies [39]. The characteristic absorption band between 1000–1150 cm^{-1} may be linked to C–O stretching vibrations of carboxylic acids, while the bands around 1200–1300 cm^{-1} are associated with N–H bending vibrations of amines [40].

Further analysis indicated that the bands within 1600–1650 cm^{-1} may correspond to stretching vibrations of primary and secondary amines [40].

The presence of carboxyl and amide groups suggests the existence of secondary amines, which are signature markers of proteins. These findings confirm that proteins or phytochemicals in the V. officinalis extract played a crucial role in the bio-fabrication of AgNPs. The identified biomolecules were found to be responsible for the capping and stabilization of the synthesized nanoparticles, further validating their role in nanoparticle synthesis and stabilization.

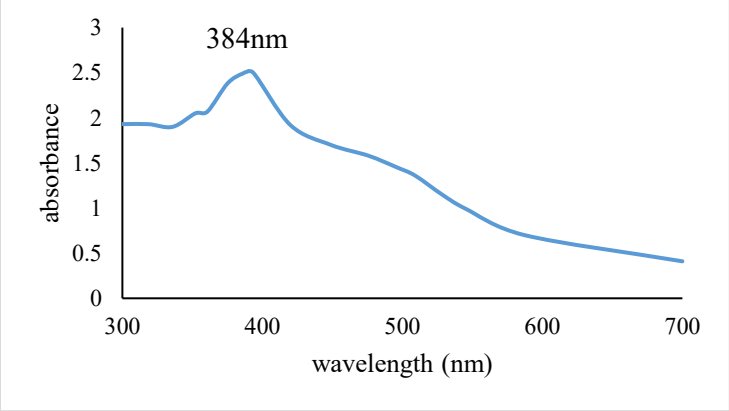

Figure 1. UV–vis absorption spectra of AgNPs synthesized from V.officinalis aqueous, after 24hr

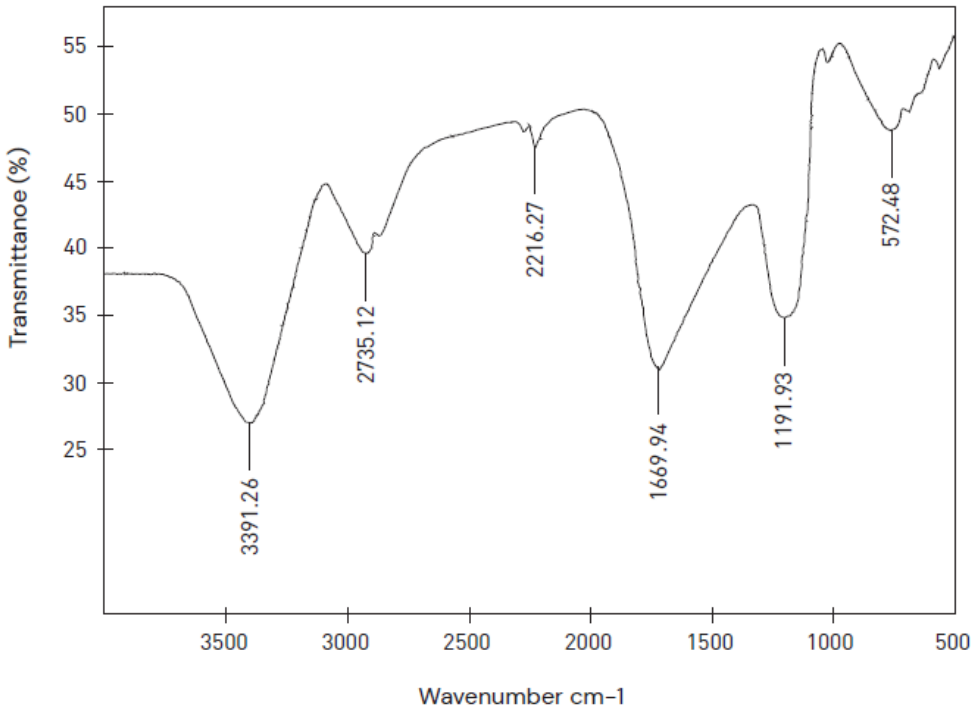

Figure 2: FT-IR spectra of AgNPs synthesis by extract of V. officinalis

Characterization by X-Ray Diffraction (XRD)

The crystalline structure of silver nanoparticles (AgNPs) synthesized using V. officinalis extract was verified through X-ray Diffraction (XRD) analysis. The diffraction peaks in the XRD pattern were analyzed and compared with standard reference data. The observed peaks at 2θ values of 38.000°, 44.160°, 64.400°, and 77.330° correspond to the (111), (200), (220), and (311) Bragg's reflections, respectively, characteristic of the face-centered cubic (FCC) crystalline structure of metallic silver (as per standard JCPDS card No. 04-0783 or 87-0597).

Figure 3 illustrates the XRD pattern of the synthesized AgNPs, with diffraction peaks recorded in the 2θ range of 10–80°. The presence of these specific peaks confirms the successful synthesis of silver nanoparticles and their crystalline nature.

The broadening of the Bragg diffraction peaks further indicated the nanoscale size of the synthesized silver particles. The average crystallite size of the green-synthesized AgNPs was calculated to be approximately 22.67 nm.

Contrarily, Gomathi et al. (2017) documented a smaller mean crystallite size of 18 nm for the synthesized AgNPs using Datura stramonium leaf extract. The observed variation in particle size across studies highlights the influence of different plant extracts and synthesis conditions on nanoparticle size and properties [41]. The XRD analysis confirms the crystalline nature of the AgNPs synthesized using V. officinalis extract, with an average crystallite size of 22.67 nm, further validating the efficiency of the green synthesis method.

Scanning Electron Microscopy (SEM) Analysis

The morphological characterization of the synthesized silver nanoparticles (AgNPs) was conducted using Scanning Electron Microscopy (SEM). Figure 4 presents the SEM image of the AgNPs synthesized using V. officinalis extract. The analysis revealed that the nanoparticles were predominantly spherical in shape and exhibited a uniform size

distribution. This uniformity in size and shape suggests the effectiveness of V. officinalis extract as a stabilizing and reducing agent during the synthesis process.

The SEM images also provide insight into the surface morphology and aggregation behavior of the nanoparticles. While the majority of the AgNPs were well-dispersed, some degree of agglomeration was observed, which may be attributed to the high surface energy and interactions among the nanoparticles. Similar observations have been reported in prior studies, where plant-based synthesis methods have led to consistent nanoparticle sizes and shapes, along with occasional agglomeration. For instance, Gomathi et al. (2017) demonstrated the synthesis of spherical AgNPs using Datura stramonium leaf extract with comparable morphology and size distribution [41]. The SEM analysis thus confirms the successful synthesis of silver nanoparticles using V. officinalis extract, with uniform morphology and a relatively small size, making them suitable for various biomedical and industrial applications. The observed size range and morphology further validate the green synthesis method's capability to produce high-quality nanoparticles with minimal environmental impact.

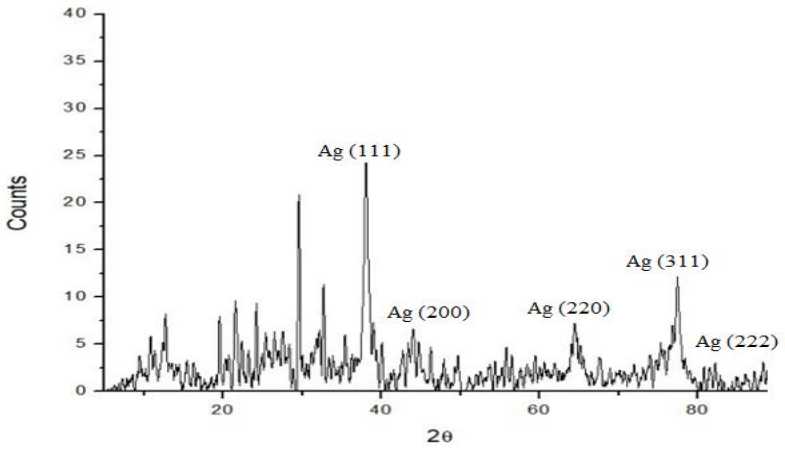

Figure 3: XRD Spectra of AgNPs using extract of V. officinalis

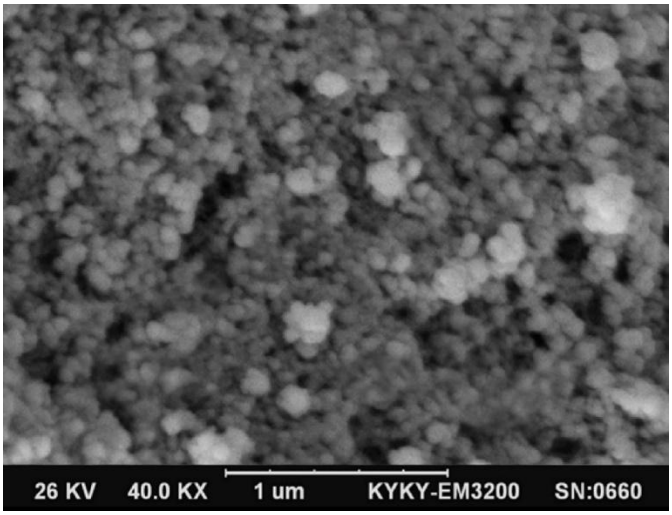

Figure 4. SEM Images of Silver Nanoparticles Formed by the Reaction of Silver Nitrate and Extract of Verbena officinalis

DLS and Zeta Analysis

After confirming the successful synthesis of silver nanoparticles through visual characteristics and the observation of a peak in the 380–420 nm range using UV-Vis spectroscopy, dynamic light scattering (DLS) analysis was conducted to validate the results. This analysis assessed the average particle size, polydispersity index (PDI), and zeta potential of the nanoparticles.

The synthesis process, carried out at room temperature, involved free silver ions and reducing agents present in Verbena officinalis extract. The extended interaction between these components resulted in the reduction of more silver ions over time. Additionally, the stabilizing compounds in extract played a key role in ensuring the stability and uniformity of the nanoparticles. The results of the particle size measurement showed that the nanoparticles ranged in size from 10 to 50 nm, with an average particle size of approximately 38 nm. The particle size distribution revealed that a significant volume percentage of particles was below 50 nm, highlighting the high desirability and uniformity of the synthesized nanoparticles (figure 5).

Zeta potential analysis provided crucial insights into the stability and interaction of the colloidal suspension (figure 6). The stability of the nanoparticles is determined by the surface charge, which in this case was negative. This negative charge creates repulsive forces between the particles, preventing aggregation and ensuring structural stability. In colloidal systems, maximizing particle repulsion is often necessary to maintain dispersion, while in some cases, minimizing these forces may promote controlled aggregation for specific applications. The balance between these forces can also influence the viscosity of the solution, making it possible to adjust its properties as needed.

The zeta potential values confirmed the high stability of the nanoparticles, attributed to the reducing and stabilizing agents in extract. These agents effectively suppressed undesirable phenomena like agglomeration, resulting in nanoparticles with good uniformity and stability. The high surface charge observed in the nanoparticles further demonstrated their stability, as stronger repulsive forces reduce the likelihood of particles sticking together. This repulsion stabilizes the system and ensures the nanoparticles remain evenly dispersed.

In conclusion, the presence of stabilizing compounds in extract not only supported the reduction of silver ions but also enhanced the stability of the nanoparticles, yielding highly uniform and stable particles with desirable properties.

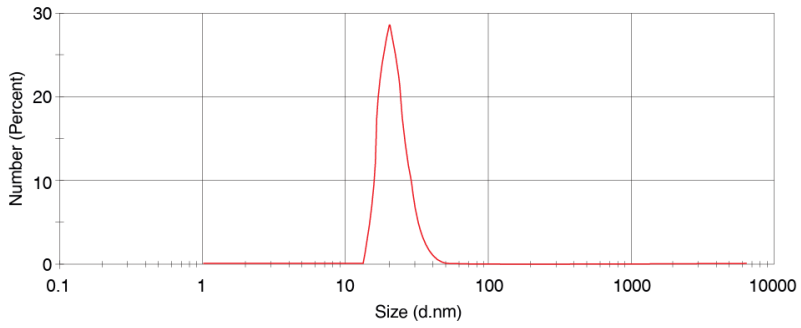

Figure 5: Diagram of nanoparticle size distribution with DLS device

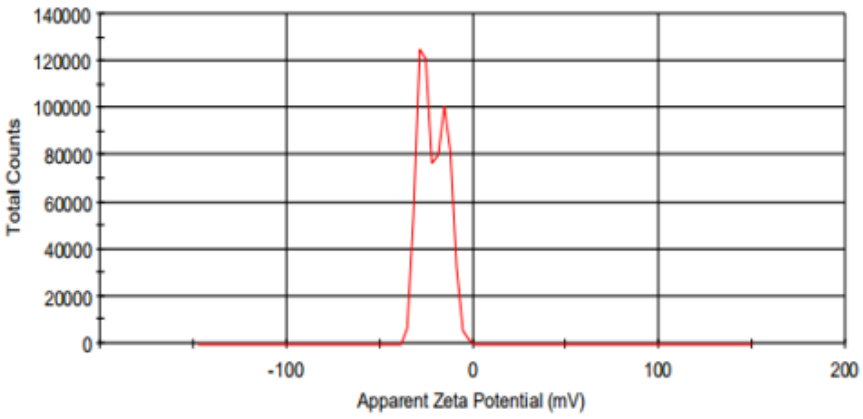

Figure 6: Diagram of apparent zeta potential of silver nanoparticles determined by DLS

CONCLUSION

This study successfully synthesized silver nanoparticles (AgNPs) using Verbena officinalis extracts through an eco-friendly green synthesis approach. UV–Vis spectroscopy confirmed successful synthesis with an absorption peak at 384 nm, characteristic of Surface Plasmon Resonance (SPR). FT-IR analysis identified functional groups such as carboxylic acids, amides, and polyphenols, confirming the role of phytochemicals in reducing and stabilizing the nanoparticles. XRD analysis indicated a crystalline face-centered cubic (FCC) structure with an average crystallite size of 22.67 nm. SEM analysis revealed predominantly spherical nanoparticles and smaller than many previously reported values, demonstrating the efficiency of V. officinalis extracts. Zeta potential analysis showed a negative surface charge, ensuring high stability by preventing particle aggregation. A low polydispersity index (PDI) further confirmed uniform nanoparticle dispersion. The synthesis mechanism was evident from the color change during the process, from milky white to dark brown, indicating the reduction of silver ions by the plant extract. Active phytochemicals like proteins and polyphenols played a critical role in reducing Ag^{+} to Ag^{0} and stabilizing the nanoparticles. These findings highlight the potential of Verbena officinalis as an efficient and sustainable source for synthesizing AgNPs. The small size, spherical morphology, and high stability of these nanoparticles make them suitable for various applications, particularly in biomedical and industrial fields.

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