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Sustainable Silver Nanoparticle Synthesis via Euphorbia Caducifolia: Exploring their Potential Application

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Abstract: *Biologically synthesized silver nanoparticles are becoming more and more popular due to their simple formation, safety, and good optical, effective antimicrobial and antioxidant activity at lower concentrations than conventional chemical pesticides. In this context, Euphorbia Caducifolia Haines, often called Snuhi, is a plant that is known for its latex, which is used to make Snuhi gel and oil. Its therapeutic qualities and traditional uses make this plant significant, underscoring its importance in a variety of treatments. The focus of this investigation is to create Ag NPs using methanolic plant extract of E. Caducifolia. FTIR and UV-VIS absorption spectroscopy, XRD, SEM, TEM techniques were employed to characterise the synthesized nanoparticles. The potential applications of Euphorbia-mediated AgNPs in optical devices, biosensing, and bioimaging are demonstrated by PL responses. Utilizing the DPPH technique, the antioxidant activity was determined and effectiveness against the plant pathogenic bacterium Staphylococcus aureus and Enterococcus faecalis was evaluated.*

Introduction

One of the foremost current fields of materials science research at the moment is nanotechnology. Recent advances in nanotechnology have been astounding, with a range of methods created that produce nanoparticles with certain sizes and shapes according to specific requirements [1].

Nanotechnology has become the vibrant discipline research. Substantial advancements have made it feasible to produce nanoparticles with precise sizes and shapes for specific applications. Biology, Chemistry, Medicine, and Materials science all make extensive utility of metal nanoparticles, especially silver nanoparticles (AgNPs) [1]. Due to the employment of intense reducing agents or harsh temperatures, traditional physical and chemical procedures for synthesizing AgNPs are frequently expensive, energy-intensive, and can produce toxic byproducts [2]. Green synthesis, on the other hand,

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has gained a lot of fascination since it offers a much more straightforward, environmentally benign, economical and non-toxic method for producing nanoparticles utilizing plant extracts by employing a bottom-up approach. Plant-based synthesis avoids the environmental and safety issues associated with traditional technique by using naturally occurring bioactive chemicals such phenols, terpenoids, sugars, and flavonoids that function as electron donors and antioxidants [3]. These naturally occurring phytochemicals act as a key player in the biosynthesis of AgNPs through sustainable and mild reaction conditions while avoiding use of harmful reagents [3-4].

A subtropical succulent blooming plant belonging to the spurge family Euphorbiaceae is *Euphorbia Caducifolia*. With around 2008 species in the kingdom of plants, *Euphorbia* is among the most diverse genera [5]. Frequently found in the dry areas of the Indian subcontinent's northwestern region, it is also referred to as the leafless milk hedge in India [6]. The sticky white latex released upon minor damage to the phylloclades constitutes a complex mixture of phytochemical constituents.

Phytochemical investigations reveal that *E. Caducifolia*latex and extracts contain diverse secondary metabolites, including terpenoids (such as euphol, tirucallol and cycloartenol), steroids, fatty acid esters, phenolic compounds, flavonoids, alkaloids, glycosides and saponins, all of which are bioactive plant metabolites with wound-healing, antibacterial, anti-inflammatory, and antioxidant qualities. [7-9]. These molecules possess functional groups (hydroxyl, carbonyl, aromatic rings) capable of electron transfer processes and molecular interactions that are central to both pharmacological and nanotechnology applications [10].

Usually, three steps are followed in the procedure of plant-assisted synthesis, we shall now discuss them in brief.

Step I: Reduction of Silver Ions

A silver precursor, silver nitrate (AgNO3) is blended with an aqueous plant extract. The extract's phytochemicals reduce the Silver(I) cation (Ag^+) to neutral silver atoms (Ag°) by giving them electrons. Phenolic chemicals, flavonoids, and terpenoids are mostly utilized in this electron donation process. The freshly created nanoparticles' surface plasmon resonance causes a noticeable colour shift in the solution, which indicates the reduction [12]. Researchers have observed that use of aqueous extracts of Euphorbia wallichiihas led to the development of crystalline AgNPs and found enormous amounts of phenols and flavonoids contributed significantly the reduction of silver ions [13].

Step II: Nucleation and Growth

Reduced silver atoms group collectively to form nuclei. As more and more Ag atoms join the clusters, the nuclei produced acts as seeds for future growth. The size of particle and distribution are demonstrated by the active phytochemical content in plant extract and rate of reduction, which in turn affects the rate of nucleation. Higher concentrations of potential reductants, majorly phenolics yields smaller, more homogeneous nanoparticles and boosts up the process of nucleation [10,12].

Step III: Capping and Stabilization

The newly synthesized AgNPs are thermodynamically unstable as they possess high surface energy thus allowing them to congregate. Flavonoids, sugars, glycosides and terpenoids are examples of phytochemicals that adhere superficially to nanoparticle, forming a protective stabilizing layer which halts aggregation, and influences their surface chemistry and morphology. Additionally, this capping is responsible for biological functions prominently, antibacterial activity [12].

By functioning as reductants and allowing electron transfer to Ag^+ ions, plant extracts have exhibited dual roles in biosynthesis of AgNPs. Various synthesis conditions, such as temperature, plant extract concentration, pH and ability to react, play an important role in controlling the reduction process, physicochemical properties, and surface stabilization of the nanoparticles. Moreover, alkaline pH and elevated temperatures typically catalyse the reduction process and produce much smaller nanoparticles [11].

Therefore, rich phytochemical profile of *E. Caducifolia* especially its phenolics, flavonoids, and terpenoids offers an efficient biochemical matrix for green AgNPs synthesis, delivering a sustainable and prominent way to synthesize nanoparticles with potential uses in the biomedical domains.

Analysts are paying greater attention to metallic nanoparticles due to the microbial resistance and silver is more significant than other metals due to its essential role as an antibacterial specialist

[14,15]. According to several studies silver nanoparticles exhibit potent bactericidal properties towards gram-positive and gram-negative bacterial species [16,17]. The synthesis, characterization and possible role of AgNPs as antimicrobials are discussed in this work.

Material and Methodology

- **Chemical and Reagents**

Every chemical used was of high quality laboratory grade and was acquired from Sigma Aldrich and Merck. Pure clinical cultures of bacteria were taken from biotechnology department of the college.

- **Isolation of Plant Extract**

Stems of *Euphorbia Caducifolia* were gathered from Botany Department, University of Rajasthan, Jaipur, India. Euphorbia stems were properly cleaned with deionised water and were completely air dried at room temperature (around 25 ± 2 °C). The shade-dried stems were processed using a mechanical grinder to produce a fine powdered material, which was then kept dry and sealed in containers until they could be processed further. 10 g of the powdered material was immersed in 100 mL methanol for 24 to 48 hours at room temperature along with periodic shaking. Whatman No. 1 filter paper was used to obtain the extract solution, the solvent was concentrated using a rotary evaporator at lowered pressure and a dry residue was obtained. The residue was diluted with distilled water and subsequently passed through a 0.45 µm membrane filter to obtain a clear extract.

- **Preparation of Silver Nanoparticles (AgNPs)**

A 1 mM $AgNO_3$ solution was prepared by dissolving 0.017 g of silver nitrate in 100 mL of distilled water. The mixture was continuously mixed and brought to a gentle boil until it dissolved fully. The reaction medium comprised 10% v/v plant extract and AgNO3 solution. The pH was lowered to 8.5 by adding 0.1 M NaOH solution. The reaction mixture was stirred and incubated at approximately 50 °C in the dark for 35 minutes. Ag NP synthesis was visually observed by the appearance of brownish coloration. To eradicate any leftover silver ions and unbound phytochemicals, the nanoparticles were centrifuged for around 15 minutes and then washed 3-4 times with distilled water. The purified Ag NPs were preserved for further characterisation and kept in amber vials.

The production of nanoparticles was verified with the development of a surface plasmon resonance (SPR) peak at about 400–450 nm [18].

- **Characterization of Silver Nanoparticles Structural analysis of the biosynthesized silver nanoparticles was performed to verify their surface chemistry, crystallinity, shape, and formation. UV–Visible Spectroscopy: A UV–Vis spectral analysis was conducted to investigate optical characteristics. Verification of nanoparticle production and long-term colloidal stability monitoring were conducted using the distinctive SPR band [19].**

A Nicolet Magna-IR 560 spectrometer was used to record Fourier-transform infrared (FT-IR) using the potassium bromide (KBr) pellet procedure technique throughout a scanning range of 4000 to 400 cm^{-1}.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis were conducted to assess the formed silver nanoparticles' surface morphology and structural properties. Samples were gold-coated before imaging for SEM analysis, and samples for TEM were made by depositing the nanoparticle on carbon-coated copper grids and then letting them air dry [20].

The phase and crystalline structure of the AgNPs were investigated through X-ray diffraction measurements conducted at 40 kV and 30 mA with Cu Kα radiation (λ = 1.5406 Å). The scan was acquired across 20°–80° in 2θ, and crystallite size was subsequently determined using the Debye–Scherrer method [21].

- **Preparation of 1mM DPPH Solution**

A Purplecoloured DPPH solution was obtained when 4mg of DPPH was taken in 200mL volumetric flask and 100 ml of methanol (99%) was added to it. The flask was vigorously stirred, wrapped in aluminium foil and stored in cool condition.

- **Preparation of Ascorbic Acid Stock Solution**

1 ml of methanol was combined with 0.5 mg of ascorbic acid to yield the standard ascorbic acid stock solution.

- **Antioxidant Activity**

The DPPH (1,1-diphenyl, 2-picryl hydrazyl) approach is often employed because of its affordability, accessibility, and reliability. Spectrophotometric evaluations of antioxidant's ability to scavenge DPPH radicals using one electron reduction form the basis of this technique. Natural antioxidants are reported in the majority of the family's members, including Euphorbia hirta [22] and E. heterophylla [23]. The UV-vis absorption maxima of DPPH is 517 nm. The colour of the solution changes when it is reduced, and a spectrophotometer makes it easy to track the reaction's development. The following protocol is commonly used to perform the DPPH assay: Sample solution (1 ml) is combined with DPPH solution (3 ml) in methanol and placed in dark for 30 minutes. The absorbance reading was used to quantify the scavenging activity and was obtained at 517 nm on spectrophotometer. The solution's colour diminishes as it reduces. The residual DPPH percentage is computed as % Antioxidant concentrations and the concentration that results in a 50% reduction in the initial DPPH concentration is known as the IC50 [24].

To determine the percentage inhibition, the following formula was used.

Percentage inhibition = [(AbsControl– AbsSample)/AbsControl] × 100 (Abs = Absorbance)

- **Antibacterial Activity**

To assess the antibacterial qualities of the crude extracts, the well diffusion approach [25] was employed. Isolates of *S. aureus* and *E. faecalis* (Gram +ve) bacteria were acquired from the biotechnology department. A Gram positive (Gram +ve)bacteria is a kind of bacteria that has cell wall made up of thick peptidoglycan. The multi-layered peptidoglycan cell wall holds the crystal violet stain and exhibits purple colour under the microscope.

Nutrient agar medium (8% Difeco Laboratories, Detroit, USA) was prepared. A stock culture of test microorganisms was kept for 48hours at 37°C in a nutrient broth (NB) medium. Each bacterial assay petri plates were filled with the nutritional agar, which was then left to solidify at room temperature. Sterilisation and cooling were applied to the agar in Petri dishes. After allowing the contents of the plates to settle, a sterilized cork boring tool was utilized to create wells on the agar medium surfaces that were roughly 5 mm apart and 3.0 mm deep. Isolates were swabbed on petri plates with the help of swab sticks. All the respective test solutions were inoculated in 50 ul volume on the holes. Tobramycin and Vancomycin was utilised as the positive control for *Staphylococcus aureus* and *Enterococcus faecalis* respectively. The inoculated plates were kept undisturbed for an hour to enable the extract to pre diffuse. The zones of inhibition were assessed in millimetres under controlled conditions at 37 °C for 24 h.

Result and Discussion

- **UV–Vis Spectroscopic Analysis**

The produced Ag nanoparticles showed an absorption peak in the 400–800 nm UV range. The UV-Vis spectra, which revealed a distinct SPR absorption band at 426.6 nm, verified the synthesis of AgNPs. This characteristic peak arises due to the resonance of free electrons in the conduction band when activated by incident light, results in decrease of oxidation state of Ag^+ ions into elemental silver. The presence, position, and sharpness of this peak validate that the biosynthesis process using *Euphorbia caducifolia* extract successfully produced stable and uniformly dispersed silver nanoparticles. Figure 1 depicts the UV-Vis spectroscopic analysis.

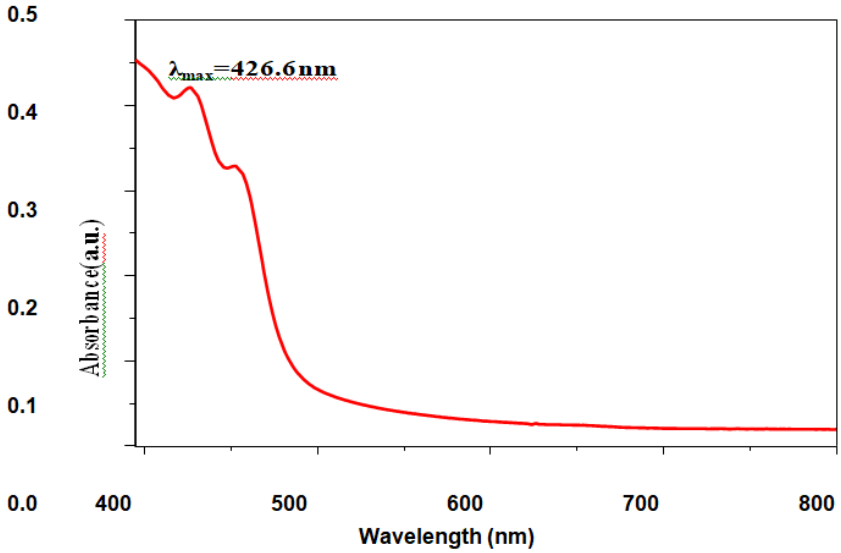

Figure 1: UV–Vis spectroscopic analysis of AgNPs The Absorbance values are listed in Table 1

Table 1: DPPH Radical Scavenging Activity (Mean ± SD), n=3) Statistical and Regression Parameters

S.No.	Concentration (mg/mL)	Absorbance(A) (Mean ± SD)	% Inhibition (Mean ± SD)
1.	4	0.812 ± 0.010	43.61 ± 0.69
2.	8	0.745 ± 0.012	48.26 ± 0.83
3.	12	0.618 ± 0.015	57.08 ± 1.04
4.	16	0.412 ± 0.011	71.38 ± 0.76
5.	20	0.294 ± 0.009	79.58 ± 0.62
	Control	1.440 ± 0.020	

- Correlation coefficient (r) 0.9739
- Coefficient of determination (R^2): 0.9485
- IC50 (mg/mL): 1.949

- **FTIR spectroscopy**

In this investigation, the FTIR spectra of the synthesised AgNPs and the plant extract from E. caducifolia were recorded in order to analyse their functional groups (Figure2). There were several shared peaks in the spectra of the plant extract and AgNPs, suggesting the existence of similar functional groups. The indication of O-H stretching vibrations may be viewed by the huge peak that both the plant extract and AgNPs displayed at 3420 cm^{-1}. Other similar peak between the plant extract and AgNPs was a brief peak at 2869 cm^{-1}. This peak is linked with the stretching vibrations of cyanide (C-N) groups which confirms the presence of nitrile functional group. Additionally, both had a prominent peak at 1662 cm^{-1}, which can be linked to C=O stretching and indicates that they possess carbonyl groups. The presence of C-H bending and C-O stretching vibrations was exhibited by additional small peaks in the plant extract at 1325 cm^{-1} and 1216 cm^{-1}, respectively. Moreover, the strength of these peaks elevated in the AgNPs, demonstrating that these functional groups may have come into contact with the surface of the NPs. Remarkably, the FTIR spectra of AgNPs displayed a small, sharp peak at 1086 cm^{-1}. The stretching vibrations of metal-oxygen (Ag-O) bonds may be responsible for this peak, which suggests the creation of AgNPs.

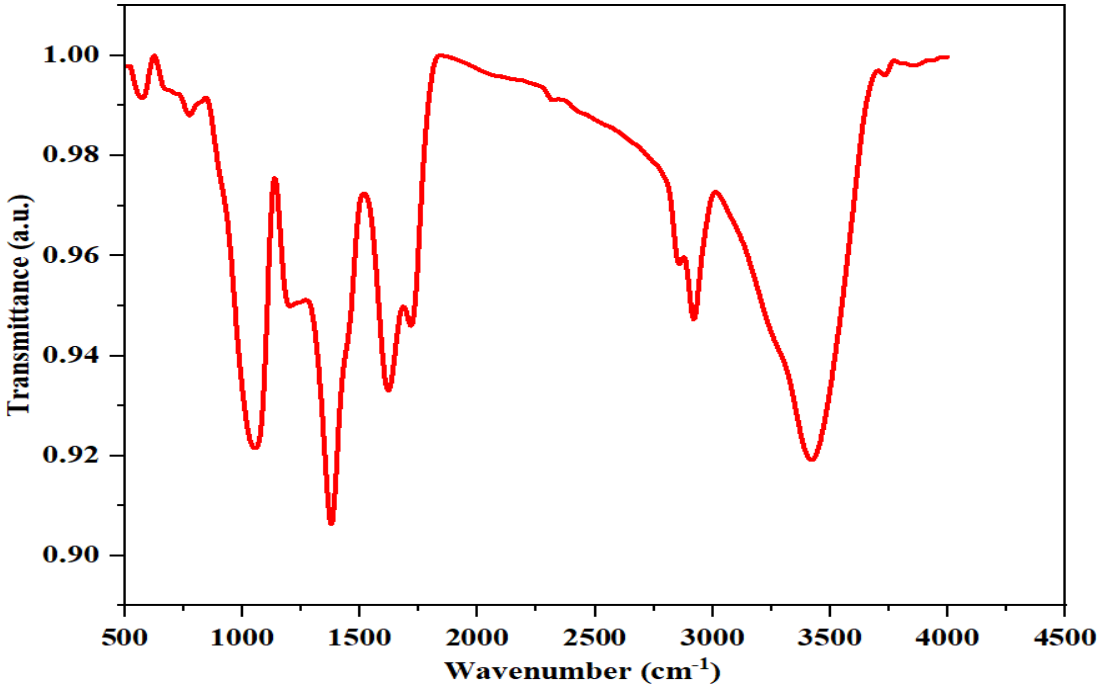

Figure 2: FTIR Spectra of *E. Caducifolia*extract and the synthesized AgNPs

Several comparable absorption bands were seen in the obtained FTIR spectra of the AgNPs and the *E. Caducifolia* plant extract. A strong band at 3420 cm^{-1}corresponds to O-H stretching vibrations of phenolic and alcoholic groups, reflecting the participation of polyphenols in the bio reduction process [26,27]. Aliphatic C–H stretching vibrations peak is observed at 2869 cm^{-1} [27]. C=O str of carbonyl or amide groups is represented by a sharp band at approximately 1662 cm^{-1}, suggesting potential involvement of proteins or flavonoids [28]. The peaks at ~1325 cm^{-1} and ~1216 cm^{-1} indicates C–H bending and C–O stretching vibrations, whose increased intensity in AgNPs suggests adsorption of phytoconstituents on the nanoparticle surface. The Ag–O stretching vibrations might be responsible for a clear peak in AgNPs at about 1086 cm^{-1}, suggesting the production of nanoparticles [29].

The FTIR applies to both the plant extract as well as the synthesized AgNPs but both have different roles. In AgNPs, FTIR confirms those functional groups which remains adhere to the surface of NP suggesting their predominant role in reduction and capping of AgNPs. In contrast, the extract identifies the major functional groups like carbonyl, phenols, proteins etc. Common peaks (~3420 cm^{-1}, ~1662 cm^{-1}) in both the spectra indicate that the phytochemicals present in the extract play a significant role in producing nanoparticles. The interaction of these biomolecules to the surface of AgNPs is demonstrated by variations in intensity or minor shifts in AgNPs spectra with respect to the extract. The Ag-O vibrations are responsible for the extra peak in AgNPs about ~1086 cm^{-1}, which supports the manufacturing of nanoparticles.

- **X-ray diffraction (XRD)**
  - **Numerous diffraction peaks at 2θ values of 38.12°, 44.28°, 64.41°, and 77.35°which matches the (111), (200), (220), and (311) planes of face-centered cubic structure (FCC) silver (JCPDS No. 04-0783) were broadcast by XRD patters. The average crystal size was calculated by applying Debye-Scherrer equation and found to be 21.6 nm. The lack of superfluous peaks remarked the phase purity of prepared AgNPs. Figure 3 shows a plot between intensity and 2θ values.**

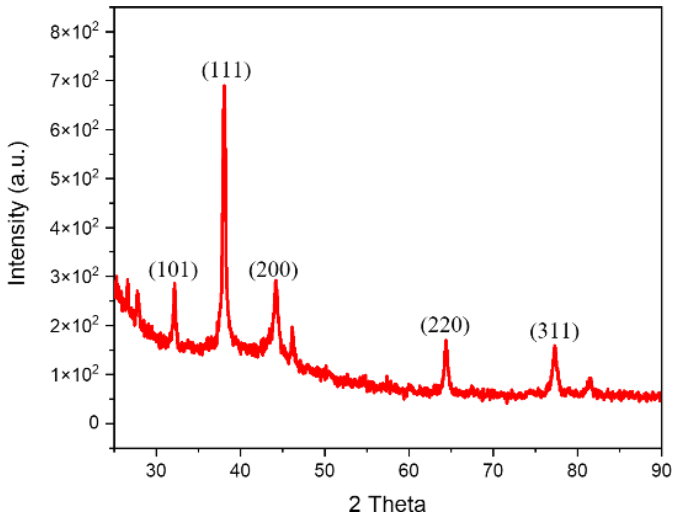

Figure 3: Plot of Intensity vs 2θ

- **SEM and TEM analysis**

SEM micrographs demonstrated mostly spherical nanoparticles with a slight degree of polydispersity (Figure 4). The particle size varied from 15 to 25 nm, and TEM analysis confirmed the spherical shape. The development of distinct lattice fringes in high-resolution TEM images (Figure 5) supported the crystalline structure of the nanoparticles in agreement with XRD data.

The obtained results exhibited that *E. Caducifolia's* stem extract functions as a successful bio reducing as well as capping agent when silver sulphate is used as a precursor in the preparation of stable and crystalline AgNPs. NPs with desirable size and crystalline characteristics are preferred for possible biomedical and catalytic utilizations.

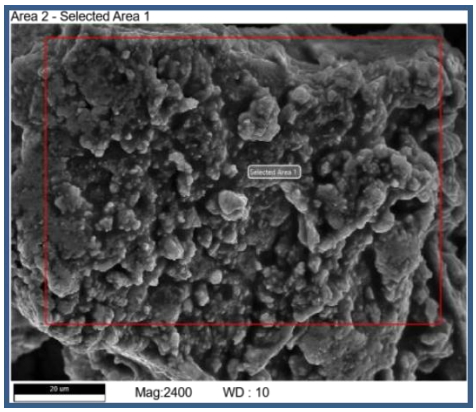

Figure 4: SEM analysis

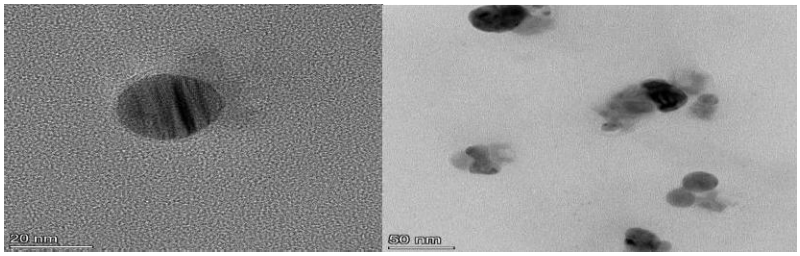

Figure 5: TEM analysis

On the basis of TEM observations, following histogram (Figure 6) has been drawn. It determines the size distribution of biosynthesized AgNPs.

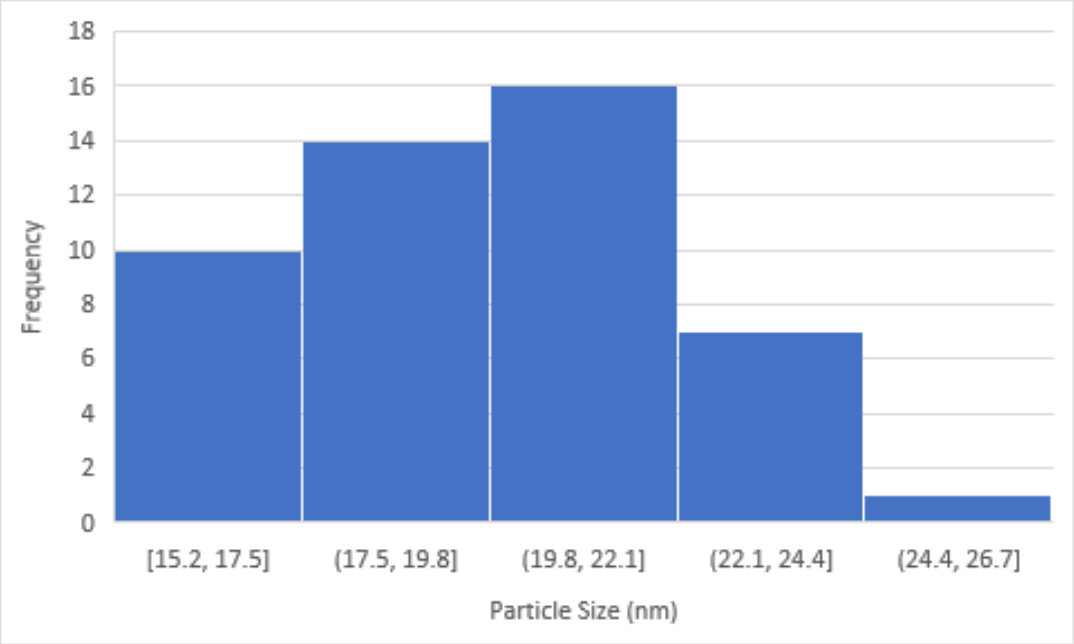

Figure 6: Size variation of biosynthesized AgNPs

Comparative Interpretation

In contrast to previously recorded plant-mediated synthesis, the AgNPs synthesized using stem extract of *E. Caducifolia*exhibits reduced polydispersity, well defined spherical morphology, a narrower particle size range (15-25nm). The comparably homogeneous size distribution improves appropriateness for biological and catalytic applications, and these results show that *E. Caducifolia* extract offers effective reducing and stabilizing phytochemicals.

- **Antioxidant Activity**

The DPPH method of testing is predicated on the idea that free radicals may acquire hydrogen from antioxidants. As a result, the reduction of DPPH radicals is closely correlated with the oxidative inhibition of the assessed samples. The presence of hydrogen donors from the antioxidant causes the DPPH radicals to transition from a purple to a yellowish hue, enabling them to become more stable [30]. The results are symbolize as IC50, which is the quantity of antioxidant that can neutralise 50% of DPPH free radicals. . Greater antioxidant activity is reflected by a smaller IC50 value. All of the extracts in this investigation exhibited reduced antioxidant activity when compared to the reference. By graphing the percentage of DPPH inhibition as a function of extract concentration, the IC50 value for each extract or reference component was ascertained. (Figure 7). The % inhibition was utilized to create a linear regression equation y=9.506x + 31.464 with an r value of 0.9739. The IC50 (x) can be calculated easily by putting y=50.

The value of x obtained is = 1.949 mg/mL

The DPPH radical scavenging assay revealed concentration-dependent antioxidant activity, with an IC_{50} value of 1.949 mg/mL derived from the regression equation (y = 9.506x + 31.464; R^2 = 0.9485). The production of nanoparticles is correlated to the antioxidant attribute. Along with scavenging DPPH, hydroxyl and phenolic containing compounds have ability to reduce Ag^+ ions to Ag^0 stabilizing the formed nanoparticles simultaneously. The mechanistic importance of phytochemicals in both nanoparticle formation and radical stabilization is thus evidenced by the moderate IC50 value. The antioxidant activity by DPPH inhibition is shown in Figure 7.

Overall Evaluation

The merged structural (SEM, XRD and TEM, FTIR) and antioxidant results exhibit that stem extract of *E. Caducifolia* is a viable bio reducing and capping agent. The overall efficacy of this ecologically friendly synthesis methodology for prospective catalytic and biomedical applications is indicated by generation of tiny (15-25 nm) AgNPs which are crystalline and comparatively monodispersed.

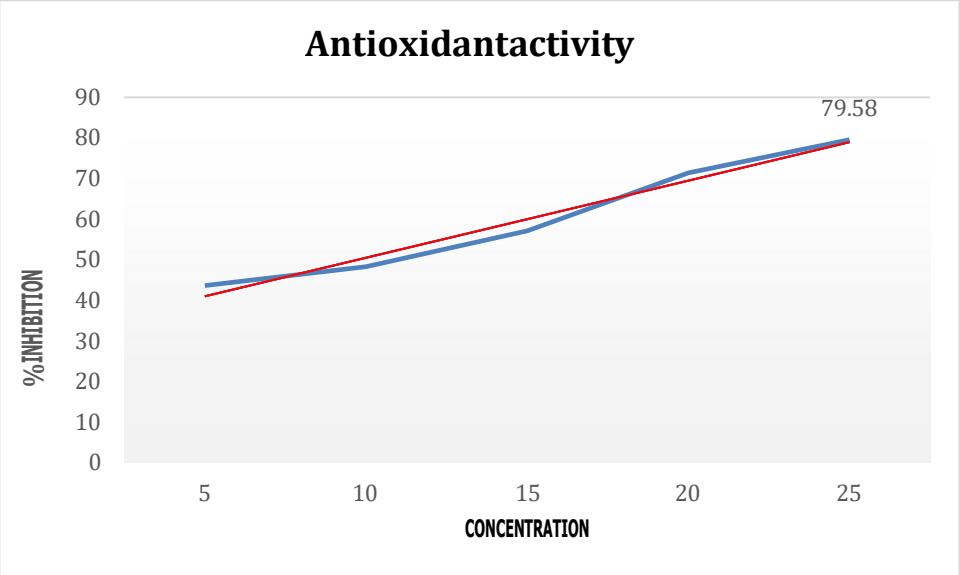

Figure 7: Antioxidant Activity by DPPH Inhibition

- **Antibacterial Activity**

Applying the agar diffusion assay, antibacterial attribute of Euphorbia- derived AgNPs was investigated against two Gram-positive pathogens, Staphylococcus aureus and Enterococcus faecalis. The inhibition zones of 22 mm for S. aureus and 21 mm for E. faecalis at 50 µg/ml were recorded for AgNPs.

The high surface-volume ratio of AgNPs, effective for Ag^+ which released and intimate engagement with bacterial cell walls is primarily responsible for their elevated activity. Silver ions are capable of passing through thick peptidoglycan layers of both pathogens but AgNPs also produce reactive oxygen species (ROS) which bind to thiol (-SH) groups of bacterial enzymes, impairing vital metabolic functions [31,32].

Furthermore, Euphorbia phytochemicals that function as stabilizing agents may work along with AgNPs to break the bacterial biofilms and increase membrane permeability [33]. The results are in close proximity with earlier observations that plant-mediated AgNPs have strong bactericidal activity against Gram-positive bacteria. The nanoparticles depicted inhibitory zones of 21.5 ± 0.5 mm for S. aureus and 19.5 ± 1.5 mm for E. faecalis (mean ± SD) at a test volume of 50 µg/ml of silver nanoparticle solution. A great consistency and steady antibacterial performance are demonstrated by the low standard deviation value.

Comparative Evaluation

AgNPs have noticeably higher antibacterial activity in comparison to the crude plant extract (50 µL at equivalent concentration), which usually results in smaller inhibition zones (about 8–14 mm in similar tests). This advancement demonstrates that the innate antibacterial capability of plant phytoconstituents is enhanced by nanoparticle production.

The AgNPs reflected equivalent antibacterial activity against S. aureus, when compared to conventional antibiotics like ampicillin or ciprofloxacin (inhibition zones normally ranging between 20–30

mm under similar assay conditions). Although antibiotics may show slightly larger zones depending on concentration, AgNPs offer the advantage of multi-targets mechanisms of action.

Mechanistic Interpretation

The enhanced antibacterial activity of AgNPs can be attributed to:

- High surface-volume ratio facilitating increased interaction with bacterial membranes
- Sustained liberation of Ag^+ ions
- Formation of reactive oxygen species (ROS)
- Binding to thiol (-SH) groups of essential bacterial enzymes
- Disruption of membrane integrity and biofilm formation

Furthermore, phytochemicals from *Euphorbia* acting as capping agents may contribute synergistically by enhancing membrane permeability and destabilizing bacterial cell walls.

Photoluminescence (PL) Spectroscopy

The emission characteristics of the produced AgNPs was investigated using photoluminescence analysis. Figure 8 illustrates the photoluminescence (PL) emission spectrum of the synthesized AgNPs recorded in the wavelength range of 300 to 900 nm. On account of the inherent fluorescence of phytochemicals including flavonoids and phenolics, the crude extract showed modest emission bands in the visible spectrum. Following nanoparticle production, AgNPs showed widened and powerful emission peaks in the 420–480 nm range with a high intensity of approximately 45,000 cps (Figure 8). The enhanced luminescence is ascribed to the surface plasmon resonance phenomenon in conjunction with electron–hole resynthesis at the surface of nanoparticle.

The PL intensity indicates that phytochemicals serve as capping agents that alter the surface electronic states of AgNPs in addition to reducing Ag^+ ions. In addition to their antibacterial and antioxidant properties, such PL responses demonstrate the potential uses of Euphorbia-mediated AgNPs in optical devices, biosensing, and bioimaging [34,35].

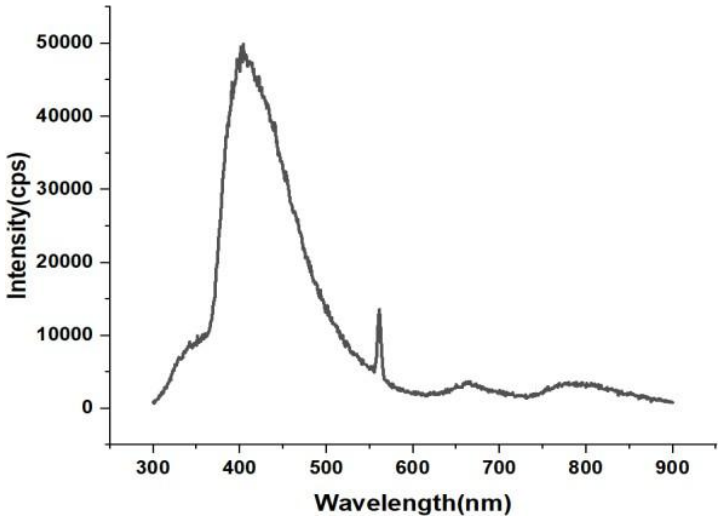

Figure 8: Plot of Intensity vs Wavelength

Conclusion

Euphorbia Caducifolia, a plant rich in terpenoids, polysaccharides, tannins, flavonoids, and phenolic chemicals, shows potential for a number of medicinal uses. High antioxidant activity may be due to increased amount of phenolic and flavonoid components. The above findings suggest that medicinal plants show significant antioxidant activity against numerous degenerative diseases. It also exhibits powerful antibacterial activity against E. faecalis and S. aureus and. The significantly larger inhibition zones produced by AgNPs compared to the crude plant extract confirm that nanoscale formulation markedly enhances antibacterial potency. The activity observed against clinically relevant Gram-positive

pathogens suggests that *Euphorbia*-mediated AgNPs possess promising therapeutic potential. Their effectiveness, combined with eco-friendly synthesis, supports their applicability as alternative or complementary antimicrobial agents, particularly in the context of rising antibiotic resistance. Further, the potential applications of Euphorbia-mediated AgNPs in optical devices, biosensing, and bioimaging are demonstrated by photoluminescence spectroscopy responses.

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