



Synthesis, Characterization, And Antioxidant Potential Of Silver Nanoparticles Of *Biophytum Sensitivum*

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Abstract

Silver nanoparticles are in high demand worldwide due to their numerous uses in biology, material engineering, and consumer goods. The study emphasized the synthesis, characterization, and study of antioxidant activity of ethanolic leaf extract of *Biophytum sensitivum* silver nanoparticles. *Biophytum sensitivum* was synthesized by employing top down technique. These nano formulation was characterized by using particle size, entrapment efficient, dissolution studies which revealed the formulation of nanoparticles with required characteristic features.

Anti oxidant potential of the nano formulation was screened using H₂O₂ and DPPH radical scavenging tests. The results indicated that the nano formulation was found to possess significant anti- oxidant activity which was better than that of crude extract. Hence the *Biophytum sensitivum* silver nanoparticles demonstrated tremendous Potential applications include biomedicine and antioxidants.

Keywords: *Biophytum sensitivum*, Nanoformulation, Entrapment efficient, Dissolution, H₂O₂, DPPH

INTRODUCTION

With several applications in the food industry, food safety, biomedicine, pharmaceuticals, water treatment, the environment, and cosmetics.[1-3] nanotechnology is a rapidly expanding scientific discipline. One of the most important developments in Using nanotechnology to address today's concerns and difficulties are particles called nanoparticles (NPs), which are less than 100 nm in size.[4] Because they have a greater surface area than volume ratio and are incredibly tiny, NPs offer superior qualities and unique architectures. Because of this, they can be utilized to produce a wide range of design materials with different qualities.[5]

On the contrary, hazardous waste products were produced during the thermal degradation, microwave irradiation, or chemical processes used to produce metal-NPs.[6] As a result, plant, fungal, and bacterial extracts are natural systems that manufacture clean, safe, and biodegradable metal nanoparticles.

Silver nanoparticles' (Ag-NPs') distinct optical, chemical, and physical characteristics have attracted attention from all over the world. They are used in many different applications, such as wound healing, biological detection, drug administration, catalysis, and antimicrobials.[7-9] The cosmeceutical industry has long acknowledged Ag-NPs because of their An vast range of pharmacological uses.[10] Unfortunately, these processes are expensive and need a lot of toxic chemical reagents, as well as intricate stages. According to certain accounts, natural resources such as microbial enzymes and Plant extracts are used to create Ag nanoparticles.[11-12] Ag-NPs were maintained in size and shape by plant metabolites, which also transformed Ag ions into Ag metals. Many plant metabolites, including proteins, sugars, terpenoids, and alkaloids, identified to be involved in the green synthesis of Ag-NPs.[13] However, because of their potent reducing abilities and the high stability of biosynthesized Ag-NPs, phenolic compounds are the main plant metabolites involved in this reaction.[14-15]

Biophytum sensitivum, a small tree in the oxalidaceae family Small and delicate, *Biophytum sensitivum* DC (Oxalidaceae) grows in Madagascar, South Asia, and Africa's tropical regions. This "little tree plant" is well-known for having an intriguing trait that makes it resemble touch-me-not plant. The herb has been used historically to treat a wide range of conditions, such as cervical spondylitis, bursitis, carpal tunnel syndrome, bone spurs, arthralgia, and arthritis.[16-18] This work used the response surface approach to examine the potential of *Biophytum sensitivum* phenolic compounds/extracts to function as a capping and reducing agent during AgNP biosynthesis in order to enhance the production conditions. The structure, morphology, antibacterial, antioxidant, and thermal stability of the resulting Ag-NPs were also investigated. In this work, the ethanolic leaf extract of *Biophytum sensitivum* was manufactured, its antioxidant capacity was defined, and its antioxidant capacity was evaluated using in vitro models.

MATERIALS AND METHODS

Materials:

In January, *Biophytum sensitivum* leaves were harvested at Vikarabad, Telangana. Analytical grade substances were employed in the investigation.

Extraction:

Once the plant material had dried, it was pulverized into fine powder. The Powdered plant material was put in a conical flask. and macerated by soaking it in ethanol. Whatman paper was used to filter the retrieved material. The ethanol was heated to 40°C using a heating mantle, and the resultant liquid was subsequently dried in a desiccator.



Figure 1: Maceration Technique

Synthesis of silver nanoparticles

To prepare a fresh *Biophytum sensitivum* leaf solution, ten grams of finely chopped, well cleaned leaves were mixed with one hundred milliliters of sterilized double distilled water. A 300-milliliter Erlenmeyer flask contains water. The concoction was then boiled for five minutes and decanted. After passing through Whatman filter paper number 1, the extract may be used within a week while being kept at -15°C . In an Erlenmeyer flask, the filtrate was mixed with an aqueous 1mM AgNO_3 solution and allowed to sit at room temperature. Therefore, the production of a brownish-yellow fluid implied that silver nanoparticles were forming. The findings demonstrated that a plant-based aqueous extract could lower aqueous silver ions, producing remarkably stable silver nanoparticles in water.[19]

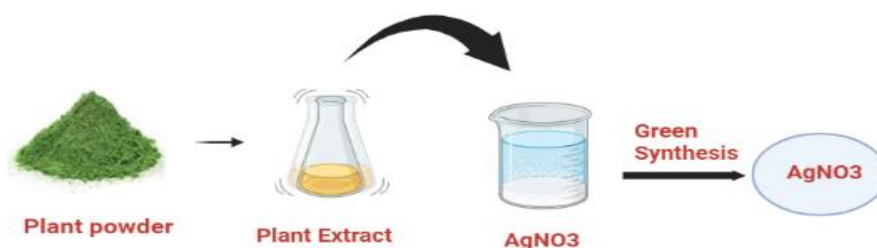


Figure 2: Synthesis of silver nanoparticles

Characterization:

The development of silver nanoparticles in leaf extract can be readily detected by UV visible spectroscopy. By periodically extracting 1 mL aliquots of the aqueous component and analyzing the solution's UV-Vis spectra, the bio-reduction of Ag^+ ions in solutions was observed. A Vasco 1301 spectrophotometer was used to measure the UV-Vis spectra of these aliquots over time in the 400–600 nm range with a 1 nm precision.

1. Particle Size Distribution (PSD)

Using dynamic light scattering, the size of the drug nanoparticles was ascertained soon after precipitation. The drug solution was diluted with filtered water to a concentration of 0.2 mg/ml before to analysis. Nine milliliters of double-distilled water were combined with one milliliter of silver nanoparticle solution in order to measure the particle size. A Microtrac particle size analyzer was used to measure the diameters of the particles.[20]



Figure 3: Microtrac particle size analyser

2. Entrapment Efficiency:

The formulation of silver nanoparticles was tested for entrapment efficiency using the centrifugation method. After adding 10 milliliters of the nanoparticle solution to a centrifuge tube, the suspension was spun for 30 minutes at 14,000 rpm. After obtaining one milliliter of supernatant, it was diluted in phosphate buffer (pH 7.4). A 254 nm wavelength UV spectrophotometer was used to find the unentrapped AEBs.

$$\% \text{ Entrapment Efficiency} = \frac{C_t - C_f}{C_t} \times 100$$

Where, C_t = Concentration of total drug

C_f = Concentration of untrapped/ free drug

3. Dissolution Studies:

Silver nanoparticle dissolving profiles were examined using the type II paddle approach, which requires dissolution equipment. In order to maintain the bath temperature at 37.0 ± 0.5 °C, the paddle rotation speed was set at 100 rpm. To the 900 milliliters of filtered water, a reservoir containing 100 milligrams of medicine powder was introduced. We repeat the dissolve test with equipment type I, a basket for raw and silver nanoparticles, respectively, to prevent the powder from floating in the water. The pH of the 900 ml PBS (phosphate buffer solution) media is 7.4. Aim for 100 rpm for both the stirring rate and the dissolution apparatus. At 10, 20, 30, 40, 50, 60, 90, and 120 minutes after the addition of silver nanoparticles

loaded with AEBS, samples should be taken out. Five milliliters of PBS should be added for each sample pullout. Once the material has been extracted, filter it and determine the absorbance at 413.[21]

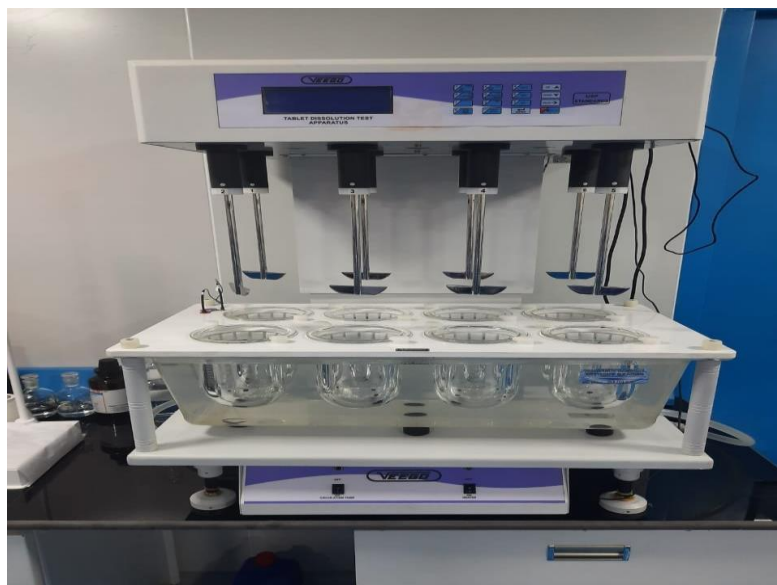


Figure 4: Dissolution Apparatus

Antioxidant activity:

1. Hydrogen peroxide (H₂O₂) radical scavenging activity assay

Per the guidelines of the Indian Pharmacopoeia 1996, a solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide was prepared. To make 200 ml of phosphate buffer (pH-7.4), 50 ml of potassium dihydrogen phosphate solution, 39.1 ml of 0.2 M sodium hydroxide solution, and adequate distilled water were added to a 200 ml volumetric flask. The free radicals were created by mixing 50 milliliters of phosphate buffer solution with the equivalent amount of hydrogen peroxide. The solution was let to rest at room temperature for five minutes following the reaction. Using 0.6 milliliters of hydrogen peroxide solution and 1ml of extract in distilled water, the absorbance at 230 nm was measured using a spectrophotometer. Hydrogen peroxide-free phosphate buffer served as the blank solution. Using the following formula, the extract's percentage of H₂O₂ scavenging was calculated.

$$\text{Percent scavenge (H}_2\text{O}_2) = \frac{A_0 - A_1}{A_0} \times 100$$

While A₀ is the absorbance of the control,

A₁ is the absorbance with the extract and standard.

2. DPPH free radical scavenging assay

The result was a methanolic solution with 0.1 mM DPPH. After 30 minutes of the fluid being left in the dark, the reaction was finished. Nine milliliters of DPPH solution and one milliliter of plant extract containing ascorbic acid were combined, and the mixture was allowed to stand at room temperature for half an hour. One milliliter of methanol and nine milliliters of DPPH solution were mixed together to act as a control. Three distinct approaches were taken to illustrate each scenario. Using methanol as a blank, the

absorbance reduction of each sample at 517 nm was determined with a UV-visible spectrophotometer. The scavenging activity was measured by the following formula:

$$\% \text{ inhibition} = \frac{Ac - At}{Ac} \times 100$$

While, Ac is the absorbance of control

At is the test sample's absorbance.

The results were given as IC₅₀ values; a lower IC₅₀ value indicates more DPPH scavenging capacity. [22]

RESULTS

Preparation of ethanolic extract of *Biophytum sensitivum*

The yield of the extract which was prepared by maceration was found to be 9.3% w/w.

Particle size determination

The formulation of silver nanoparticles loaded with AEBS has a particle size within the range of 43nm and 0.043µm.

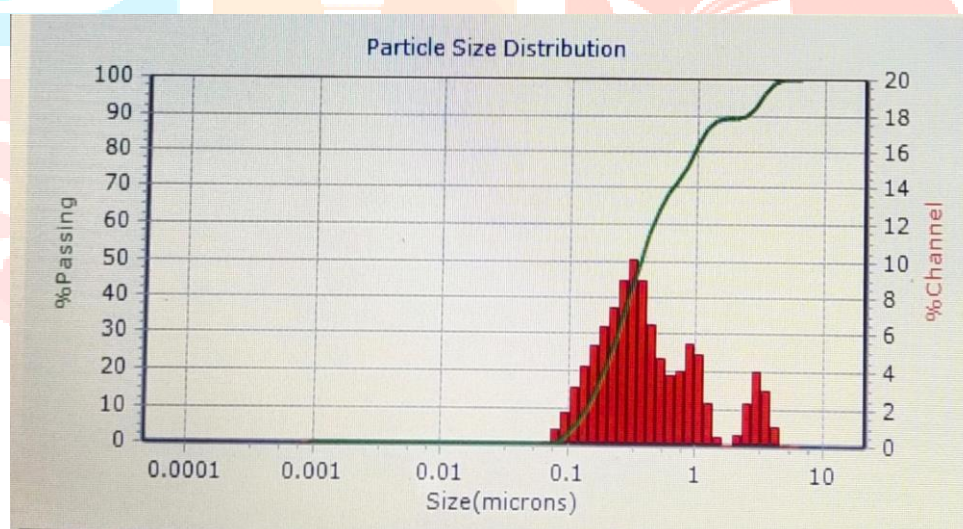


Figure 5: Particle size determination

Entrapment efficiency

Percentage of entrapment efficiency obtained for AEBS loaded Silver nanoparticles of formulation was obtained in the range of 83.03.

Dissolution studies

The percentage drug release of AEBS loaded Silver nanoparticles of formulation were mentioned in the table

Table 3: Percentage of drug release of AEBS loaded Silver nanoparticles of Formulation and extract

Formulation	% Drug release
Extract	72.81
Formulation	84.88

ANTIOXIDANT ACTIVITY

Hydrogen Peroxide Scavenging Assay:

The percentage inhibition of plant extract, formulation and ascorbic acid was found to be increased with increase in their concentrations. The IC50 value of the formulation (35.08) found to be better than that of crude extract (39.13). The hydrogen peroxide scavenging potential of the formulation was found to be very near to that of standard ascorbic acid (36.80).

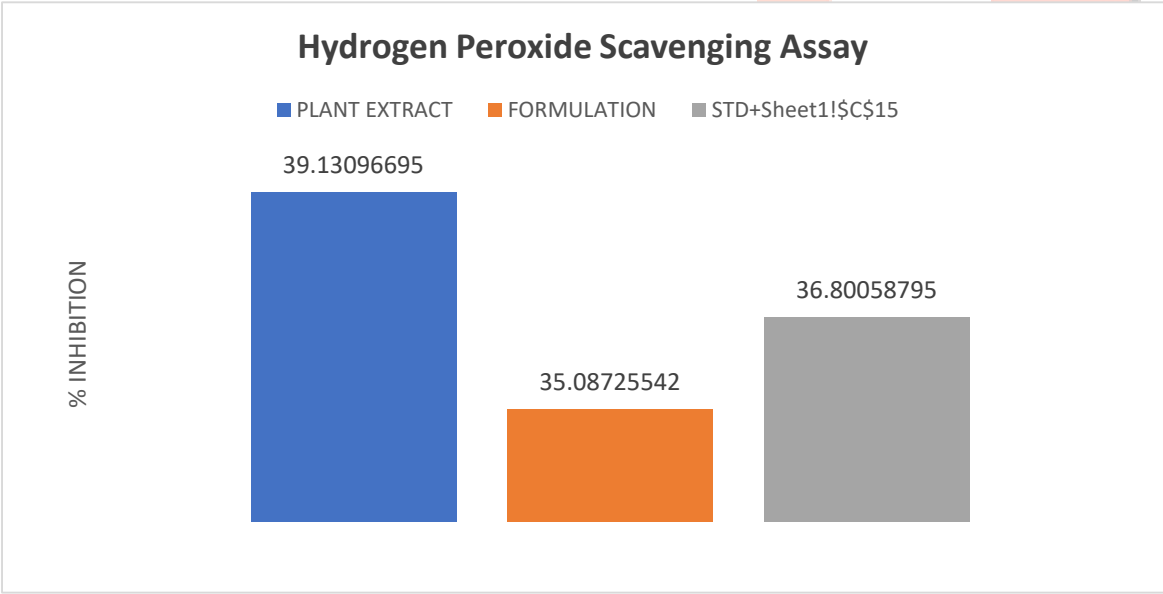


Figure 6: Hydrogen peroxide scavenging acitivity of nano formulation, crude extract of *Biophytum sensitivum*.

DPPH Free Radical Scavenging Assay:

The percentage inhibition of plant extract, formulation and ascorbic acid was found to be increased with increase in their concentrations. The IC₅₀ value of the formulation (34.68) found to be better than that of crude extract (36.24). The hydrogen peroxide scavenging potential of the formulation was found to be very close to that of standard ascorbic acid (32.009).

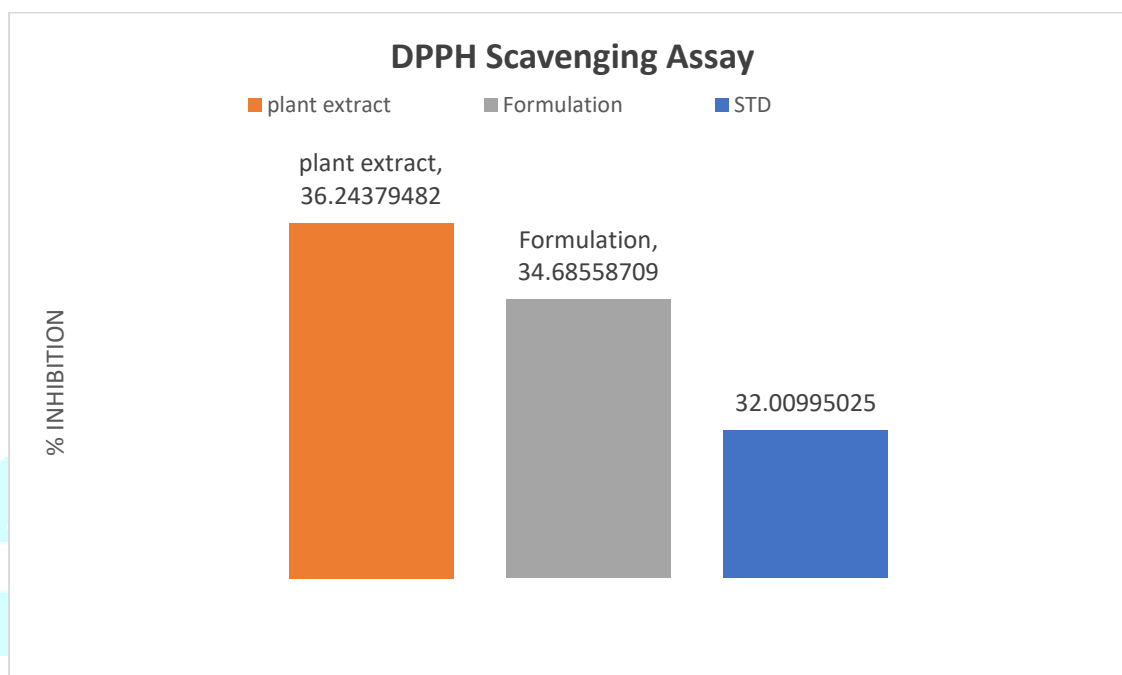


Figure 7: DPPH scavenging activity of nano formulation, crude extract of *Biophytum sensitivum*.

Discussion

A new method of drug delivery called nanoformulations has the potential to revolutionize medication delivery and improve patient outcomes. They can be used to treat a variety of ailments and offer many advantages over conventional drug delivery techniques. Nanoformulations are crucial in many domains, especially pharmacy, medicine, agriculture, and material science, due to their unique properties and benefits over traditional formulations, including enhanced solubility, bioavailability, targeted drug delivery, controlled release, and reduced toxicity.

The silver nanoparticles' particle size *Biophytum sensitivum*'s ethanolic extract was measured at 0.043 μ m (43 nm). Nanoparticles fall within the designated size range of 1 to 100 nm as a result.

Entrapment efficiency in silver nanoparticles entrapped The The ethanolic leaf extract of *Biophytum sensitivum* was reported to be 83.03% effective for nanoprecipitation. The study proved the entrapment of the extract was very significant and falls in the permissible limits of entrapment.

For the extract and formulation, the dissolve tests using six baskets of dissolving equipment showed 72.81% and 84.88% drug release, respectively. Smaller particles have a larger surface area and can release the drug more quickly, which leads to greater dissolution than larger particles. This is because drug solubility is

directly proportional to particle surface area, as per the Noyer- Whitney equation. The nanoformulation should therefore dissolve more quickly than the crude extract. The current study's findings are consistent with the Noyer- Whitney equation.

DPPH and hydrogen peroxide were among the methods used to measure antioxidant activity. A purple solution can be bleached using the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) to determine a natural substance's ability to donate electrons. According to the approach, the DPPH is scavenged when an antioxidant is added to the DPPH solution. A correlation exists between the degree of color change and the content and efficacy of antioxidants. A notable decrease in The reaction mixture's absorbance indicates that the chemical under test has a high capacity to scavenge free radicals. In the present investigation, the hydroalcoholic extract exhibited a favorable association with the total phenolic content and a much higher inhibition %. By comparing hydrogen to a free radical, the experiment's findings show that the phytochemical components in the plant extract are suitable for gauging the possible harm.

Normal concentrations of hydrogen peroxide are found in food, water, air, plants, microbes, and the human body. Rapid H₂O₂ breakdown into oxygen and water can produce hydroxyl radicals (OH·), which can damage DNA and induce lipid peroxidation. The presence of phenolic groups in the hydroalcoholic extract of *Biophytum sensitivum* leaf may have contributed to the successful scavenging of hydrogen peroxide, which was then neutralized into water by the donation of electrons.

Conclusion

The study proved that the nano formulation of ethanolic extract of *Biophytum sensitivum* possess the required characteristic features and better antioxidant potential compared to the crude extract. Further studies are required to isolate the phytoconstituents and test their efficacy in suitable models.

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Conflict of interest

Authors of this work declared no disputes or conflicts in the publication of this manuscript

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