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Photobiogenic Mediated Silver Nanoparticles Synthesis and Its Antimicrobial Activity

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Silver nanopartilces was synthesized by photobiogenic method using Euphorbia hirta aqueous extract under the bright sunlight in 25 min at pH 7.5. Euphorbia hirta extract acts as a capping agent of silver nanoparticle synthesis. The synthesized Eh-AgNPs were characterized by UV-vis spectroscopy, HR-TEM, and zeta potential. The SPR peak of Eh-AgNPs was produced at 424nm. The Eh-AgNPs was found to be spherical shape with average size of 12nm. The synthesized Eh-AgNPs was highly stabled in the liquid medium. The antibacterial activity of different concentration of Eh-AgNPs against both Gram positive and Gram negative pathogenic bacteria were analyzed by the well diffusion assay method. Eh-AgNPs inhibited the growth of both group of bacteria in the concentration manner. The maximum zone of inhibition15mm, 22mm, 19mm and 20mm were obtained at 100µg/ml concentration for Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa respectively. The synthesized Eh-AgNPs has well antibacterial activity against both group of bacteria particularly it has maximum activity against pathogenic bacteria such as Pseudomonas aeruginosa, and Staphylococcus aureus.

Keywords: Silver nanoparticles, Euphorbia hirta, Sunlight irradiation, Green synthesis, Physicochemical characterization, Antibacterial activity.

1. Introduction

Nanoparticles has special attraction duet to its size, shape, adaptable surface modification,

bioconjugation, optical, electrical and thermal properties1. Among the different metal nanoparticles, silver nanoparticles have huge potential due to having high surface area to volume ratio and it has vast applications from antimicrobial to photo catalyst2,3. The silver nanoparticles can be synthesized by physical, chemical and biological methods. The physical and chemical method of nanoparticles synthesis is required high energy, pressure, sophisticated instruments, and involves toxic chemicals. Biological synthesis of nanoparticles has gained attention compare to physical and chemical methods. The bioinspired syntheses of nanoparticles using plants are the non toxic, easily available, low cost, environmentally friendly methods4. Previously, several research works have been published on biogenic synthesis of metal nanoparticles using plant extract. Recently, Shereen et al., 2024 reported that plant extract preparation and green synthesis of silver nanoparticles using Swertia chirata5. Ejaz et al., 2024 explained the characterization, synthesis, and biological activities of silver nanoparticles produced via green synthesis method using Thymus vulgaris aqueous extract6. Silver ions and silver-based compounds are highly toxic to microorganisms including 16 major species of bacteria. This makes silver an excellent choice for multiple roles in the medical field. Silver is generally used in the nitrate form to induce antimicrobial effect, and when nanoparticles are used, there is a huge increase in the surface area to be in contact with microbial cells7.

The present research work describes the biogenic synthesis of silver nanoparticles using Euphorbia hirta aqueous extract under the sunlight in biological pH. Euphorbia hirta is belongs to the family of Euphorbiaceae, which is traditionally using medicinal plant for treatment of various disorders and diseases8. Euphorbia hirta has several potential pharmacological activities including antioxidant, anti-inflammatory and anticancer9, wound healing potential10, anthelmintic, antidipsogenic, antiarthritis11, antibacterial12, antianaphylactic13, anxiolytic14, antidiabetic15 and hepatoprotective16. The synthesized silver nanoparticles were characterized by UV-vis spectroscopy, HR-TEM, zeta potential. Further, Antibacterial activity of the synthesized Eh-AgNPs was analyzed using the well diffusion assay method. The antibacterial activity of different concentration of Eh-AgNPs was analyzed against both Gram positive and Gram negative bacteria. Eh-AgNPs inhibited the growth of bacteria in the concentration manner. Particularly, the synthesized Eh-AgNPs has excellent antibacterial activity against pathogenic bacteria such as Pseudomonas aeruginosa, Staphylococcus aureus. Previously several authors explained the antimicrobial activity of silver nanoparticles synthesized by plants extract. Crisan et al., 2024 reported that In vitro antimicrobial activity of silver nanoparticles against selected Gram-negative and Gram-positive pathogens17. Holubnycha et al., 2024 explained that the antimicrobial activity of two different types of silver nanoparticles against wide range of pathogenic bacteria18.

2. Materials and Method

Materials

Silver nitrate was obtained from Sigma Aldrich, USA. Muller Hinton Agar medium was purchased from Himedia, India. Sodium hydroxide was obtained from SRL India.

Preparation of Euphorbia hirta Aqueous Extract

Euphorbia hirta was collected from the herbal garden, University of Madras, Chennai. 35% of aqueous extract of Euphorbia hirta was prepared using sterile distilled water, clean morter and pestle. The extract was filtered with whatman No:1 filter paper and stored at 4ºC for silver nanoparticles synthesis.

Synthesis of Silver Nanoparticles

50ml of Silver nitrate solution (1mM) was taken in a clean conical flask, in which 2.5ml of Euphorbia hirta aqueous extract was added and pH of the solution was adjusted to 7.5 using sodium hydroxide solution. The solution mixture was stirred in magnetic stirrer and kept under the bright sun light; the colorless Silver nitrate solution was changed to yellow colour in 25 minutes that indicates the synthesis of Silver nanoparticles. It is stored at 4ºC for further characterization and applications studies.

Characterization of Eh-AgNPs

UV-visible Spectrophotometer Analysis

The synthesized Eh-AgNPs 3ml was taken in a clean quartz cuvette, the surface plasmon resonance (SPR) peak of Eh-AgNPs was analyzed using Shimadzu UV 1601spectrometer, Japan and the SPR peak reading was recorded and graph was plotted using origin software.

High Resolution - Transmission Electron Microscopy (HR-TEM) Analysis

The size and shape of Eh-AgNPs was analyzed using HR-TEM (FEI TECHNAI G2 MODEL T-30-S-TWIN, USA). 5µl of Eh-AgNPs was placed in a copper grid and allowed it to dry in room temperature. Then the grid was examined in HR-TEM operated at 200kV to measure the size of the synthesized nanoparicles.

Zeta Potential Analysis

The net charge of synthesized Eh-AgNPs was estimated using Nano Series (Malvern Instruments, United Kingdom). Eh-AgNPs solution was taken in an electrode cuvette without air bubbles and placed in the sample holder and the instrument was run at 100VA at 25°C and 232 kcps with the duration of 60 seconds.

In-vitro Hemolytic Assay

The hemolytic activity of silver nanoparticles was analyzed using human red blood cells by In-vitro hemolytic assay19. The blood sample was collected and appropriate anticoagulant was added into it. The red cells suspension (5%) was prepared from the collected blood sample using saline. The different concentration (20, 40, 60, 80 and 100µg/ml) of silver nanoparticles were allowed into the RBCs suspension and incubate at 37ºC for 1h. Then, the samples were centrifuged and the supernatant was used for the hemolysis estimation at 570nm in UV-visible spectroscopy.

In-vitro Antibacterial Activity

Antibacterial activity of synthesized Eh-AgNPs was analyzed by Muller Hinton Agar well diffusion method20 against Gram positive (Enterococcus faecalis, Staphylococcus aureus) and Gram negative (Escherichia coli, Pseudomonas aeruginosa) pathogenic bacteria. The bacteria

were grown in Nutrient broth for 24 hours. Muller Hinton Agar (MHA) (3.8gms/100ml) was prepared by the sterile distilled water and transferred to sterile petriplates. About 1.5 × 106 CFU/mL suspensions of each test bacteria were inoculated in surface of MHA by sterile swab. Then the following concentration of Eh-AgNPs (40, 60, 80 and 100µg/mL) was added into the well of agar plates. Plates were incubated for 24h at 37°C. The bactericidal activity of Eh-AuNPs was detected by measuring the zone of inhibition in millimeter (mm). Streptomycin and Ampicillin (30µg) were used as a control for Gram positive and Gram negative bacteria respectively.

Statistical Analysis

All the data were evaluated using the statistical software SPSS/16. Hypothesis-testing methods included one-way analysis of variance (ANOVA) followed by least significant difference test. P-values less than 0.05 were considered statistically significant. All the results were expressed as mean ± standard deviation (n = 3).

3. Results and Discussion

Synthesis of Eh-AgNPs

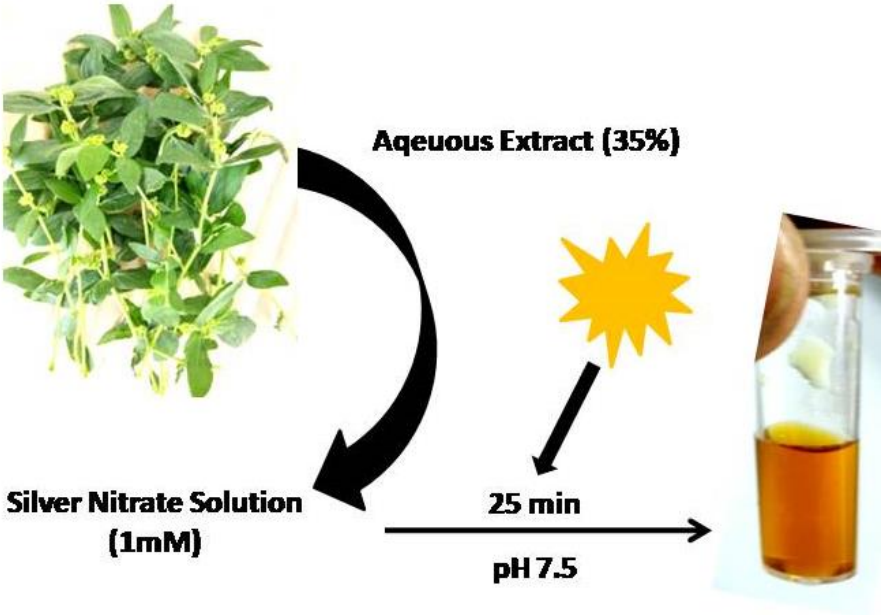

Figure 1: Schematic representation of synthesis of Eh-AgNPs by photobiogenic method under the bright sunlight irradiation.

The present work describes the Euphorbia hirta aqueous extract capped biogenic synthesis of silver nanoparticles under the bright sunlight. The colourless silver nitrate solution was changed to yellow colour after adding the Euphorbia hirta aqueous extract in 25 minutes at pH:7.5 (Figure 1). Euphorbia hirta aqueous extract acts as a capping agent and the sunlight act as a catalyst in the siver nanoparticles synthesis. This biogenic and sunlight mediated synthesis

of silver nanoparticles is a cost effective, facile, and non toxic eco-friendly method. The physical and chemical methods are costly, require sophisticated instruments, toxic chemical were used for nanoparticles synthesis21, 22.

Physico-chemical Characterization of Eh-AgNPs

UV-Visible Spectroscopy

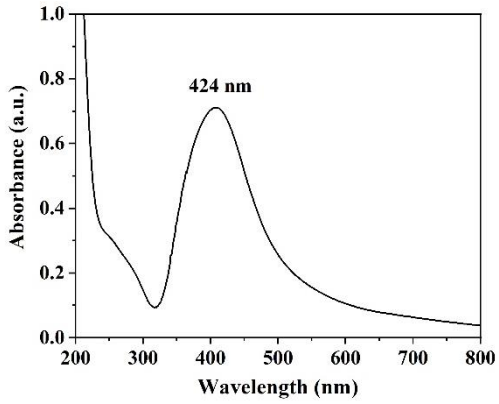

Figure 2: UV-visible spectroscopy analysis of Eh-AgNPs

The UV-visible spectroscopy is an early characterization technique to validate the nanoparticles synthesis. Generally, The AgNPs are showed SPR peak between 400 to 450nm23. The synthesized Eh-AgNPs showed narrow, single SPR peak at 424nm that confirmed the synthesis of silver nanoparticles (Figure 2). The obtained SPR peak exhibited in narrow, single peak that means the particles are in small size and homogenous. The morphology of metal nanoparticls are depending on the extract concentration, composition, pH, temperature and reaction time24,25.

Zeta Potential Analysis

The surface net charge of Eh-AgNPs was estimated by zeta potential and it was found to be -20.7mV (Figure 3). The zeta potential is determined from the electrophoretic mobility of siver nanoparticles by its degree of repulsion between adjacent, similarities charged particles 26. This strong negative charge of Eh-AgNPs is prevented the aggregation of particles, and maintain the stability in the liquid medium for long duration27.

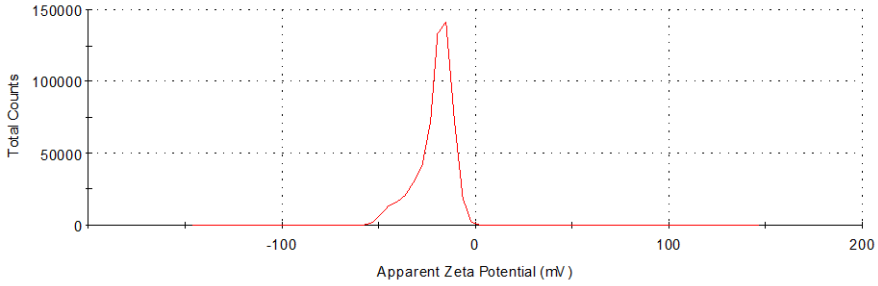

Figure 3: Zeta potential of Eh-AgNPs was found to be -20.7mV.

HR-TEM Analysis of Eh-AgNPs

The size and shape of Eh-AgNPs was analyzed through the HR-TEM. The Eh-AgNPs were spherical in shape with monodispersity. The particle size was between 9 to 21nm, and the average particles size was found to be 12nm (Figure 4). The detection of morphology of nanoparticles is very important in biological applications. A small size nanomaterials is easily penetrates the cell membrane and it can be used for various biomedical applications.

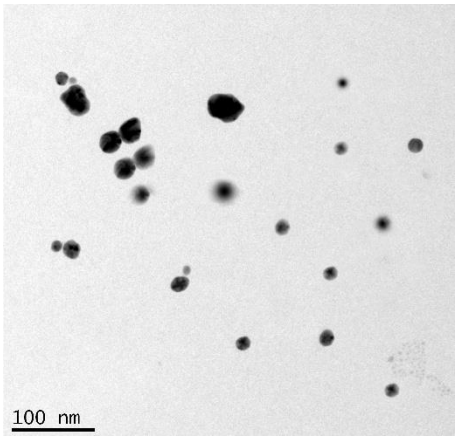

Figure 4: The morphology of Eh-AgNPs were analyzed by HR-TEM.

Hemolytic assay

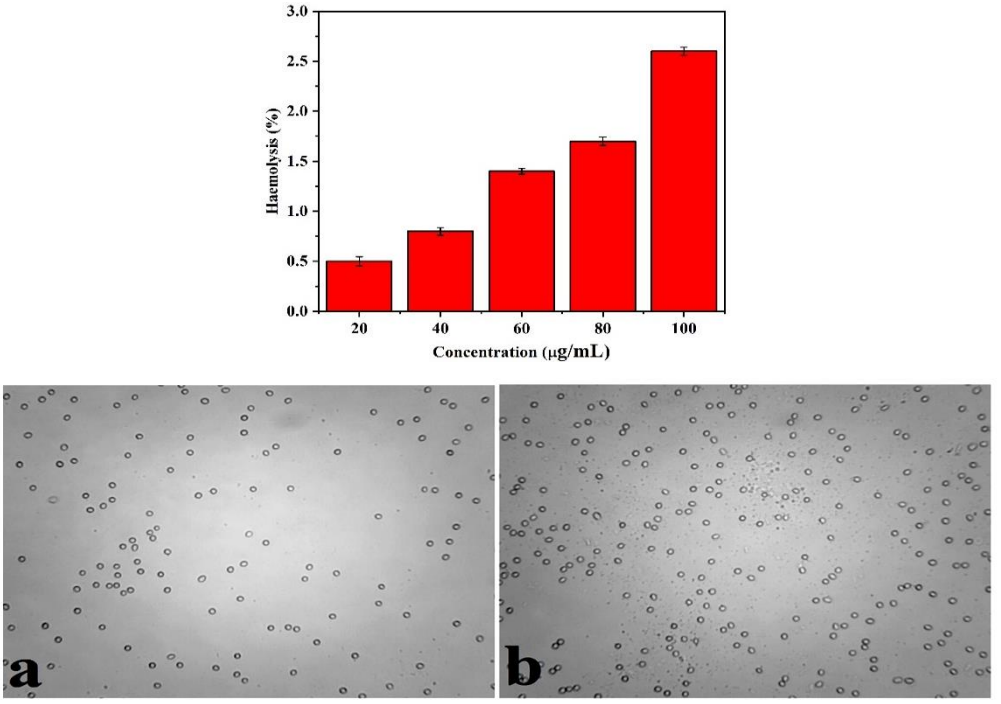

Figure 5: The hemolytic assay of Eh-AgNPs and its corresponding morphology of RBCs.

The hemocompatibility of Eh-AuNPs was analyzed in human RBCs. The different concentration of Eh-AuNPs was incubated with human RBCs and hemolysis (%) was estimated by UV-visible spectroscopy (Figure 5). About 0.5%, 0.8%, 1.45%, 1.7%, and 2.6% of hemolysis was observed for 20, 40, 60 80 and 100µg/mL concentration of Eh-AuNPs. The hemolysis (%) was depended on the concentration of the Eh-AuNPs, but the maximum tested concentration of Eh-AuNPs (100µg/mL) induced normal acceptable level of hemolysis (<5%). Therefore, Eh-AuNPs is having fine hemocompatibility property upto 100µg/mL. According to the ASTM (American standard for testing materials) upto 5% of hemolysis is considered as hemocompatible. Previously Muthukumarasamyvel et al., 2017 reported hemocompatiblity of cholic acid capped AuNPs (DCaC- AuNPs, DCaDC- AuNPs, DCaLC- AuNPs) in human RBCs. AuNPs treated human blood sample was produced 1.90, 1.97, and 2.09%, hemolysis in the respective order at 120µg/mL concentration28. Ramadurai et al.,2010 explained that the CuNCs treated RBCs, 0.5% of haemolysis was observed for a minimum concentration of CuNCs (4 µg/mL), 4.7% of haemolysis was observed for 16 µg/mL and 7.5% of haemolysis was obtained for maximum concentration of 22 µg/mL respectively29.

The hemocompatibility of Eh-AuNPs was further confirmed through the morphological analysis of RBCs. The normal RBCs is oval or biconcave shape30. There was no significant morphological change observed in Eh-AuNPs treated RBCs. Eh-AuNPs treated RBCs exhibited normal morphology like untreated control RBCs morphology and RBCs was appeared in disperse manner without any aggregation (Figure 5).

In-vitro Antibacterial Activity

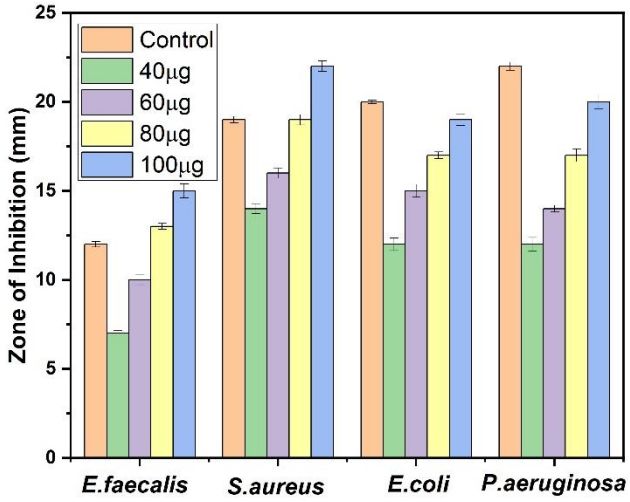

Figure 6: Antibacterial activity of Eh-AgNPs pathogenic bacterias (Zone of inhibition in millimeter)

Antibacterial activity of the Eh-AgNPs was tested using a well diffusion assay method. The antibacterial activity of different concentration of Eh-AgNPs against both Gram positive and Gram negative bacteria are shown in the figure 6. Eh-AgNPs inhibited the growth of bacteria in the concentration manner. The maximum zone of inhibition15mm, 22mm, 19mm and 20mm were obtained at 100µg/ml concentration for Enterococcus faecalis, Staphylococcus aureus,

Escherichia coli, Pseudomonas aeruginosa respectively.The synthesized Eh-AgNPs has excellent antibacterial activity against pathogenic bacteria such as Pseudomonas aeruginosa, Staphylococcus aureus. AgNPs will lead to high antimicrobial activity as compared with bulk silver metal31. The mode of action of AgNPs against bacteria is not completely understood yet, However, several hypotheses are explaining the antibacterial activity of silver nanoparticle by generation of reactive oxygen species, release of Ag + ions from AgNPs denaturize proteins by bonding with sulfhydryl groups and attachment of AgNPs on bacteria and subsequent damage to bacteria32,33,34.

4. Conclusion

The AgNPs was synthesized in a cost effective, eco-friendly, photobiogenic method using Euphorbia hirta aqueous extract under the bright sunlight irradiation. The synthesized Eh-AgNPs was exhibited in spherical shape with average size of 12nm. Eh-AgNPs has high stability with strong negative surface net charge (-20.7mV). Eh-AgNPs inhibited the growth of both Gram positive and negative pathogenic bacteria in the concentration manner. However, Eh-AgNPs has excellent antibacterial activity against pathogenic bacteria such as Pseudomonas aeruginosa and Staphylococcus aureus. Hence, this synthesized Eh-AgNPs can be used in pharmaceutical and biomedical industries for preparation of bactericidal cream for treating these above listed microorganisms.

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