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Algal Extract Mediated Silver Nanoparticles Characterization and Antibacterial Activity

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Silver nanopartilces was synthesized by green chemistry biogenic method using Dyctyota dichotoma aqueous extract under the bright sunlight in 20 min at pH 7.5. Dyctyota dichotoma aqueous extract acts as a capping agent in nanoparticle synthesis. The synthesized Dd-AgNPs were characterized by UV-vis spectroscopy, HR-TEM, DLS, zeta potential and FT-IR. The SPR peak of Dd-AgNPs was produced at 422nm. The Dd-AgNPs was found to be spherical shape with average size of 24nm. The synthesized Dd-AgNPs was highly stabled. The antibacterial activity of different concentration of Dd-AgNPs against both Gram positive and Gram negative bacteria were analyzed by the agar well diffusion assay method. Dd-AgNPs inhibited the growth of bacteria in the concentration manner. The maximum zone of inhibition12mm, 19mm, 17mm and 18mm were obtained at 100µg/ml concentration for Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa respectively. While comparing with control antibiotic, the synthesized Dd-AgNPs has excellent antibacterial activity against Gram positive pathogenic bacteria such as Enterococcus faecalis, Staphylococcus aureus.
Keywords: Silver nanoparticles, Dictyota dichotoma, Sunlight irradiation, Green synthesis, Physicochemical caharacterization, Antibcaterial activity.

1. Introduction

Nanotechnology is a promising field that addresses to the challenges of traditional medicine. Nanoparticles has special attention duet to size, shape, adaptable surface modification,

bioconjugation, optical, electrical and thermal properties[1]. Nanoparticles within the size range of 1-100nm can be utilized in biomedical applications. Among the different metal nanoparticles, silver nanoparticles have great potential due to having high surface area to volume ratio and it has diverse applications from antimicrobial to photo catalyst[2,3]. The nanoparticles can be synthesized by physical, chemical and biological methods. Biological synthesis of nanoparticles has gained attention compare to physical and chemical methods[4]. The bioinspired synthesis of nanoparticles using plants are the non toxic, easily available, low cost, environmentally friendly methods[5]. A physical and chemical method of nanoparticles synthesis is required high energy, pressure, sophisticated instruments, and involves toxic chemicals[6]. Previously, several research works have been published on synthesis of metal nanoparticles using medicinal plant and plant components extract. Recently, Selvaraj et al., 2024 reported silver nanoparticles synthesis using Tabebuia aurea leaf extract for efficient water treatment[7]. Mejía-Méndez et al.,2024 published green synthesis of silver nanoparticles with extracts from Kalanchoe fedtschenkoi: characterization and bioactivities[8].

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 cells[9]. The present research work describes the biogenic synthesis of silver nanoparticles using Dyctyota dichotoma aqueous extract under the sunlight in biological pH. Dictyota dichotoma (Hudson) Lamoroux is brown seaweed and distributed worldwide from temperate to subtropical regions. The species was first described by Hudson (1762) from Walney Island in Lancashire, United Kingdom[10]. The polysaccharide of this seaweed has potential biological activities such anticoagulant, antithrombotic, anti-inflammatory, antitumoral, contraceptive, apoptotic, antioxidants and antiviral[11]. The synthesized silver nanoparticles were characterized by UV-vis spectroscopy, HR-TEM, DLS, zeta potential and FT-IR. Further, Antibacterial activity of the synthesized Dd-AgNPs was analyzed using the agar well diffusion assay method. The antibacterial activity of different concentration of Dd-AgNPs were analyzed against both Gram positive and Gram negative bacteria. Dd-AgNPs inhibited the growth of bacteria in the concentration manner. While comparing with control antibiotic, the synthesized Dd-AgNPs has excellent antibacterial activity against Gram positive pathogenic bacteria such as Enterococcus faecalis, Staphylococcus aureus. Previously several authors explained the antimicrobial activity of silver nanoparticles synthesized by plants extract. Naim et al., 2024 reported that Biosynthesis, antimicrobial and in vitro antiproliferative activities of silver/silver chloride nanoparticles from mixed fruit extracts of Capsicum frutescens and Tamarindus indica[12]. Ali et al., 2024 explained that Effect of biosynthesized silver nanoparticle size on antibacterial and anti-biofilm activity against pathogenic multi-drug resistant bacteria[13]. Alnehia et al., 2024 published Phyto-mediated synthesis of silver-doped zinc oxide nanoparticles from Plectranthus barbatus leaf extract: optical, morphological, and antibacterial properties[14].

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 Dyctyota dichotoma Aqueous Extract

Dyctyota dichotoma was collected from Mandapam coastal region, Ramanathapuram district, Tamil Nadu, India. The contaminants were removed from the sample by washing with seawater, fresh water and distilled water respectively. 30% of aqueous extract of Dyctyota dichotoma was prepared using sterile distilled water, clean morter and pestle. The extract was filtered with whatman No:1filter paper and stored at 4ºC for silver nanoparticles synthesis.

Synthesis of Silver Nanoparticles

100ml of Silver nitrate solution (1mM) was taken in a clean conical flask, in which 1.5ml of Dyctyota dichotoma 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 turned to yellow colour in 20 minutes that indicates the formation of Silver nanoparticles. Further, the Silver nanoparticles synthesis confirmed by the surface plasmon resonance (SPR) peak analysis in UV-visible spectrophotometer. It is stored at 4ºC for further characterization and applications studies.

Characterization of Dd-AgNPs

UV-visible Spectrophotometer Analysis

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

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

The morphology of silver nanoparticles was analyzed using HR-TEM (FEI TECHNAI G2 MODEL T-30-S-TWIN, USA). 4µl of sample was placed in a copper grid and allowed it to dry at room temperature. Then the grid was examined in HR-TEM operated at 200kV for morphological analysis of nanoparicles.

Dynamic Light Scattering (DLS) Analysis

In the liquid medium, the hydrodynamic diameter of nanoparticle size was analyzed using Zeta Nano Series (Malvern Instruments, United Kingdom). Synthesized silver nanoparticles was placed in the sample holder using cuvette and the instrument was run at 100VA at 25°C and 232 kcps with the duration of 60 seconds.

Zeta Potential Analysis

The net charge of synthesized Dd-AgNPs was estimated using Nano Series (Malvern Instruments, United Kingdom). The silver nanoparticles 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.

Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

Dyctyota dichotoma algae and synthesized silver nanoparticles were separately ground with potassium bromide and made as a pellet then the presence of chemical functional group in algae and nanoparticles was analyzed using FT-IR spectrophotometer (Jasco 5300, USA).

In-vitro Antibacterial Activity

Antibacterial activity of synthesized Dd-AgNPs was analyzed by well diffusion method[15] against Gram positive (Enterococcus faecalis, Staphylococcus aureus) and Gram negative (Escherichia coli, Pseudomonas aeruginosa) pathogenic bacteria. Muller Hinton Agar (MHA) (3.8gms/100ml) was prepared using sterile distilled water and transferred to sterile petriplates. The bacteria were grown in Nutrient broth for 24 hours. About 1.5×10^6 CFU/mL suspensions of each test bacteria were inoculated in surface of MHA by sterile swab. Then the different concentration of Dd-AgNPs (40, 60, 80 and 100µg/mL) was impregnated into well (6 mm diameter) of agar plates. Plates were incubated for 24h at 37°C. The antibacterial activity of Dd-AuNPs was calculated by measuring the zone of inhibition in millimeter (mm). All antibacterial assays were performed with 3 replications. Streptomycin and Ampicillin (30µg) 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 Dd-AgNPs

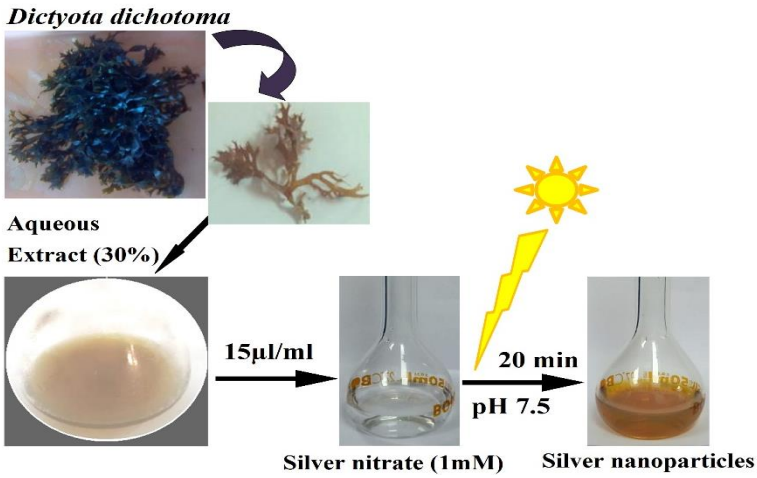

Figure 1: Schematic representation of synthesis of Dyctyota dichotoma aqueous extract capped silver nanoparticles under the bright sunlight irradiation.

The present research work explains about the Dyctyota dichotoma 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 Dyctyota dichotoma aqueous extract in 20 minutes at pH:7.5 (Figure 1). Dyctyota dichotoma aqueous extract acts as a reducing and capping agent and sunlight act as catalyst in nanoparticles synthesis. This green chemistry and sunlight mediated synthesis of nanoparticles is a cost effective, facile, and non toxic eco-friendly method[16]. The physical and chemical methods are costly, toxic chemical were used for nanoparticles synthesis and need sophisticated instruments for nanoparticles synthesis[17,18].

Physico-chemical Characterization of Dd-AgNPs

UV-Visible Spectroscopy

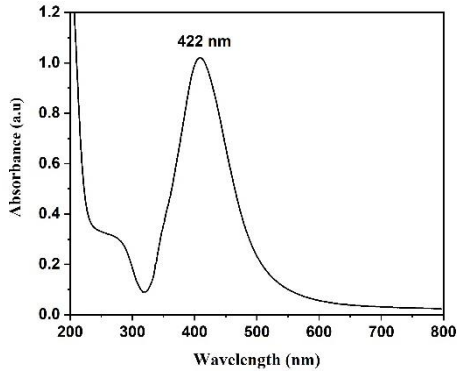

Figure 2: UV-visible spectroscopy analysis of Dd-AgNPs

The UV-visible spectroscopy is a initial characterization technique to confirm the nanoparticles synthesis. Generally, The AgNPs are showed SPR peak between 400 to 450nm[19]. The synthesized Dd-AgNPs showed SPR peak at 422nm that confirmed the synthesis of silver nanoparticles (Figure 2). The obtained SPR peak exhibited in narrow, single peak that means the particles is in small size and homogenous. The morphology of metal nanoparticls are depending on the extract concentration, composition, pH, temperature and reaction time[20,21].

Dynamic Light Scattering (DLS) Analysis

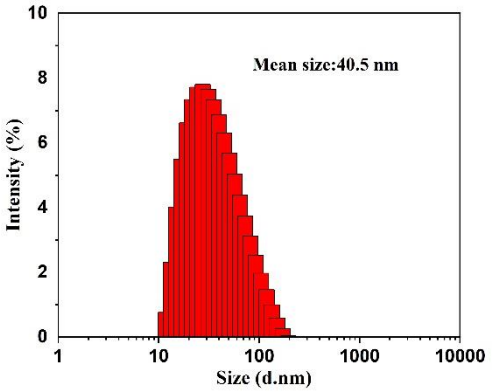

Figure 3: DLS analysis of Dd-AgNPs produced average size of 40.5nm

The hydrodynamic diameter of Dd-AgNPs was calculated by DLS method. The average size of the Dd-AgNPs in the liquid medium was found to be 40.5nm (Figure 3). Dd-AuNPs size was based on measuring the time dependent fluctuation of scattering of laser light by NPs undergoing Brownian movement[22].

Zeta Potential Analysis

The surface net chage of Dd-AgNPs was calculated by zeta potential and it was found to be -36.3mV (Figure 4). The zeta potential is determined from the electrophoretic mobility of nanoparticles by its degree of repulsion between adjacent, similarities charged particles[23]. This strong negative charge of Dd-AgNPs is prevented the aggregation of particles, and maintain the stability for long duration[24].

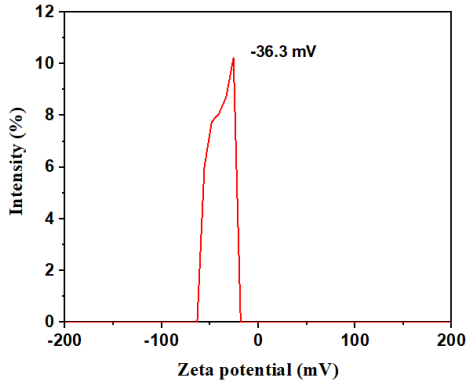

Figure 4: Zeta potential of Dd-AgNPs was found to be -36.3Mv

HR-TEM Analysis of Dd-AgNPs

The morphology of Dd-AgNPs was analyzed through the HR-TEM. The Dd-AgNPs were spherical in shape with monodispersity. The particle size was between 10 to 40nm, and the average particles size was found to be 24nm (Figure 5). 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.

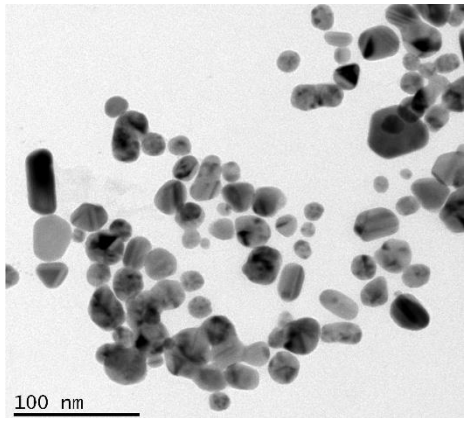

Figure 5: The morphology of Dd-AgNPs were analyzed by HR-TEM.

FT-IR analysis

Figure 6a, Dyctyota dichotoma extract showed peak at 1016cm^{-1}, 1112cm^{-1}, 1409cm^{-1}, 1654cm^{-1}, 2937 cm^{-1}, and 3304cm^{-1}. A peak at 1016cm^{-1} and 1112cm^{-1} is assigned for C-N stretching vibration of primary amines, a peak at 1409cm^{-1} and 1654cm^{-1} corresponds to carbonyl or carboxylic (C=O) stretching bands of peptide linkages (stretching of amides), 2835cm^{-1} corresponds to N-H group. The band at 2947cm^{-1} corresponds to asymmetric stretching of C-H bonds. A peak with centre at 3304cm^{-1} corresponds to O-H stretching of hydroxyl group. In figure 6b, the Dd-AgNPs exhibited peak at 1640cm^{-1} corresponds for carboxyl stretching bond (C=O), and 3418cm^{-1} corresponds to O-H group[25,26]. The absorption peak from 1409cm^{-1} to 1654cm^{-1} of Dyctyota dichotoma extract combined together and exhibited a single peak in AuNPs at 1640cm^{-1}. The amine and carboxyl group present in the Dyctyota dichotoma extract is disappeared in the Dd-AgNPs FT-IR spectra. It is responsible for reduction of silver ions to silver nanoparticles. The carboxyl group present in the extract is responsible for the stability of the synthesized silver nanoparticles.

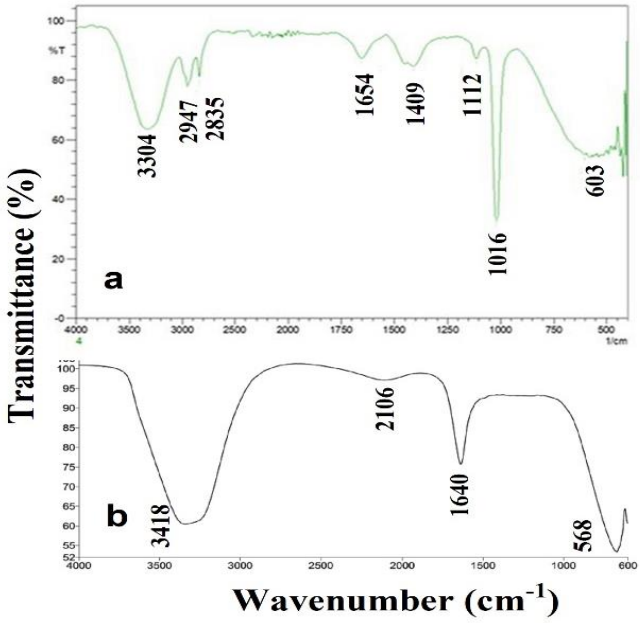

Figure 6: FT-IR analysis of Dyctyota dichotoma extract and Dd-AgNPs

In-vitro Antibacterial Activity

Antibacterial activity of the synthesized Dd-AgNPs was analyzed using the agar well diffusion assay method. The antibacterial activity of different concentration of Dd-AgNPs against both Gram positive and Gram negative bacteria are shown in the figure 7. Dd-AgNPs inhibited the growth of bacteria in the concentration manner. The maximum zone of inhibition12mm, 19mm, 17mm and 18mm were obtained at 100µg/ml concentration for Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa respectively. While comparing with control antibiotic, the synthesized Dd-AgNPs has excellent antibacterial activity against Gram positive pathogenic bacteria such as Enterococcus

faecalis, Staphylococcus aureus. AgNPs will lead to high antimicrobial activity as compared with bulk silver metal[27]. 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 bacteria[28,29,30].

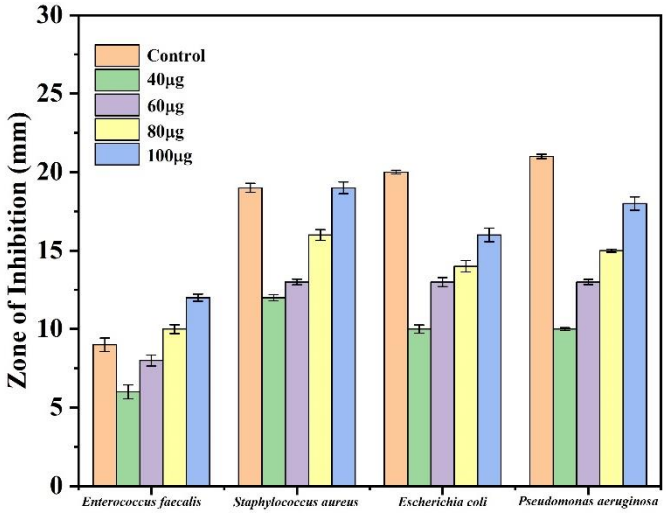

Figure 7: Antibacterial activity of Dd-AgNPs against Gram positive and Gram negative bacteria (Zone of inhibition in millimeter)

4. Conclusion

The Dd-AgNPs was synthesized in a cost effective, eco-friendly, facile biogenic method using Dyctyota dichotoma aqueous extract under the bright sunlight irradiation. The synthesized Dd-AgNPs was exhibited in spherical shape with average size of 24nm. Dd-AgNPs has high stability with strong negative surface net charge (-36.3mV). Dd-AgNPs inhibited the growth of both Gram positive and negative pathogenic bacteria in the concentration manner. However, Dd-AgNPs has excellent antibacterial activity against Gram positive pathogenic bacteria such as Enterococcus faecalis, Staphylococcus aureus. Hence, this bactericidal activity of Dd-AgNPs can be used in pharmaceutical and biomedical industries for preparation of antibacterial cream for treating this above listed microorganism.

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