GREENSYNTHESIS, CHARACTERIZATION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED WITH NIGELLA SATIVA SEED EXTRACT

Abstract

In this study, a novel technique for synthesizing silver nanoparticles (AgNPs) prepared from Nigella Sativa (NS) extract is presented. Because NS functions as a very effective stabilizing and reducing agent due to highly metabolized substances such flavonoids, terpenoids, proteins, etc., NS extract in the process is more favorable than other methods. These substances, which have reducing and stabilizing characteristics, cause the formation of AgNPs. Different characterizing techniques, such as UV-Vis, X-ray diffraction, and Fourier transform infrared spectroscopy, were used to examine the as-obtained AgNPs (FTIR). According to XRD data, well-crystallized nanoparticles of lower sizes were formed. 450 nm was discovered to have the highest UV-Vis absorption. Research has been done on the antioxidant and antibacterial properties of silver nanoparticles.

Keywords: Silver Nanoparticles, XRD, UV, FTIR, Antioxidant and Antibacterial activity.

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I. INTRODUCTION

Engineering a functional system at the molecular level is known as nanotechnology. The science that deals with the ability to control and modify matter on scales ranging from less than a nanometer to 100 nm is known as nanotechnology. The precise manipulation of matter at close to the atomic and molecular level is the primary requirement in nanotechnology [1]. The capacity to see nanoscale materials has made a wide range of companies and scientific efforts possible [2]. Nanotechnology can be used for a variety of applications, including the delivery of drugs, the creation of fabrics, molecular manufacturing, micro/nano electromechanical systems, etc [3]. Because it is simply a collection of techniques that allow modification of properties at a scale [4]. Nanoparticles are particles with an interfacial layer around them and measuring between 1 and 100 (nm) in size. The interfacial layer is a crucial component of nanoscale matter and has a fundamental impact on all of its characteristics. In most cases, ions, inorganic molecules, and organic molecules make up the interfacial layer. The stabilizers, capping and surface ligands, or passivating agents that cover inorganic nanoparticles are organic compounds [5]. A small object that behaves as a single entity in terms of its attributes and mobility is known as a particle [6]. Due to their ability to contain their electrons and induce quantum effects, nanoparticles frequently have unexpected optical features. For instance, in solutions, gold nanoparticles have a deep-red to black appearance [7]. Yellow gold and grey silicon nanoparticles are both red in colour. Unlike gold slabs, gold nanoparticles melt at lower temperatures [8]. Nanoparticle-based materials absorb solar energy at a significantly faster rate than continuous sheets of thin material film [9]. Silver nitrate is a precursor to many compounds of silver, including the silver compounds used in photography [10]. AgNO3 is quite stable when exposed to light, unlike silver halides, which are utilized in photography because of their sensitivity to light [11]. Medical and industrial applications are two areas where silver nitrate is frequently used. Many additional silver compounds that are employed are precursors to silver nitrate. Thus, making additional silver compounds is one of the uses of silver nitrate. A particularly caustic substance is silver nitrate. This is a step in the creation of various silver salts. Silver nitrate is also used to make colloidal silver compounds that are applied to medicine. Silver halides, which are utilized in photographic emulsions, are created using silver nitrate. Additionally, this is utilized in laboratories for volumetric analysis to find halides, thiocyanates, etc [12].

II. EXPERIMENTAL DETAILS

1. **Materials:** From the Coimbatore neighborhood market, we purchased the fresh seeds for NS (Kalonji seeds). A select group of chemicals, including pure ethanol, acetone, and silver nitrate (AgNO3), were purchased from the reputable Precision Scientific Co., Coimbatore. Throughout the entire investigation, glassware and distilled water (DW) have been utilized regularly as needed.

2. ***Nigellasativa*** **extract preparation:** Before beginning the procedure, distilled water is used to thoroughly clean the nigella sativa seeds. The seeds are finely ground and turned into powder. 400ml of deionized water are mixed with 10g of powdered seeds, and the mixture is held in a magnetic stirrer. The combination was heated until it reached boiling point (1000C), the nit was cooled to room temperature and filtered through Whatman (Whatman Grade 1) paper before being stored.

3. **Synthesis of silver nanoparticles using *Nigellasativa* extract:** AgNO3 solution 0.1mM was made. The AgNO3 solution and Nigella sativa extract (10ml and 11ml) were combined, and the mixture was then put in the magnetic stirrer. The solution changes colour from white to light brown after being heated for two hours at 800C. For 24 hours, they are stored in dim conditions at room temperature. Due to the reduction of silver ions and production of silver nanoparticles, the solution turns a dark brown colour. For 15 minutes, the nanoparticles are centrifuged at 2500 rpm. The contaminants are eliminated using distilled water and ethanol. Ina hot air oven, silver nanoparticles are deposited on a glass plate and heated for four hours to 90°C. After that, the characteristics of the solid silver nanoparticles are studied [13]. Figure (a) shows that the synthesis of silver oxide nanoparticles.

4. **Characterization:** XRD analysis revealed the crystal structure of silver oxide nanoparticles. Calculations of structural factors such strain, dislocation density, and crystal size were made using the XRD pattern. The common crystalline structure is 42.11nm. Using SEM images, the material's surface morphology was made clear. When using the UV-Visible analysis, the optical band gap energy is calculated to be 1.44 eV. The antibacterial study reveals that silver oxide inhibits the growth of streptococcus bacteria [14].

5. **Antioxidant Assay**

   - **DPPH Free Radical Scavenging Assay:** The anti-free radical activity of silver nanoparticles made with Nigella Sativa seed extract was measured using1, 1-diphenyi-2-picryl-hydrazil (DPPH). A methanol solution of DPPH (10mg/250 mL) was mixed with various concentrations of the existing silver nanoparticles (60, 90, 120, and 150 mg/mL). After shaking, the mixture was left to incubate for 30 minutes at room temperature in a dark area. The absorbance of each mixture was then measured at 417 nm. A control measurement was made of DPPH absorbance, which includes no sample. Three duplicates of the experiment were run. The natural antioxidant ascorbic acid was used [15]. Using the following formula, the proportion of free radial scavenging was determined:

     DPPH free radical scavenging (%) $= \frac{\text{control}-\text{test}}{control} \times 100$ (A)

6. **Antibacterial Activity:** To evaluate the antibacterial activity, agar diffusion was used. By bacterial stock culture inoculation of nutritional broth media (E. coli), the bacteria were received and grew for 18 hours at a 37% concentration. Agar plates were produced for the aforementioned media. Each plate was injected with germs that had been swabbed into the sterileplates18 hours prior. Create the five boreholes. Pour the extract in the followingratio: 10, 20, 30, and 40 ml. All of the plates were incubated for 24 hours at 37 °C, and the inhibitory zone's diameter was measured in cm. To ascertain the antibacterial activity and minimal inhibitory concentrations of plant extracts against Gram Positive and Gram Negative bacteria, the agar well diffusion method was used [16]. In tests with various microbes, the extracts demonstrated antibacterial properties.

III. RESULTS AND DISCUSSION

1. **UV-Visible spectroscopy (UV-Vis):** To confirm the production of NPs in aqueous solution, UV-Vis spectroscopy was used. In an aqueous solution, UV-Vis can be utilized to gather specific information about the size, shape, and stability of NPs. When Nigella Sativa seed extract was introduced to the AgNO3 solution, AgNO3 was transformed into AgNPs. When the hue of the solution changed from light brown to dark brown after a short period of time and full reduction of Ag+ to Ag was accomplished in 24 hours, NP formation could also be seen visually. According to Figure (b), the absorption peak of AgNPs ranges from 330 to 800 nm, with a noticeable peak occurring between 433 and 450 nm. The dominant peak in samples a, b, c, and d was absorbed at 441, 450, 441, and 433 nm. Using the following formula, the band gap energy was determined:

   Band gap energy = $\frac{1240}{\lambda}$ eV (B)

   The bandgap energy of the samples a and b are respectively 2.80eV.

2. **XRD:** The X-ray diffraction pattern of silver nanoparticles is shown in figure(c). Table (1) shows that the XRD pattern demonstrated that the Silver nanoparticles are crystalline, and the diffraction peaks at 2θ of angles 19.688^0, 21.765^0, 24.299^0, 29.757^0, $31.937^{0,}32.778^0$, 35.452^0, 39.312^0, 40.227^0, $41.989^{0,}42.824^{0,}43.473^0$, 46.284^0, 47.835^0, 49.787^0, 53.888^0 and 55.296^0 which corresponded to (111), (102), (020), (211), (113), (122), (220), (213), (131), (311), (302), (024), (320), (133), (322), (323) and (331) orientation planes for Orthorhombic structure. It closely resembles the typical JCPDS card number (00-001-0856). It is found that crystals are 25.33 nm in size on average. The following equations were used to derive the structural characteristics of silver nanoparticles from the XRD pattern [17]. Scherrer's equation was used to determine the crystalline size.

   Crystalline size $D = \frac{k\lambda}{\beta Cos\theta}$ (C)

   Where, D- particle size in nm, λ- X-ray wavelength, β- FWHM, θ- Bragg's angle of reflection, and $\boldsymbol{\theta}$ - Angle of diffraction (degree).

3. **Fourier Transform Infrared Spectroscopy (FTIR):** Figure (d) shows that the FTIR analysis of silver nanoparticles due to the presence of an amide with a C=O bond, the absorbance peak at 1649 cm^{-1} is present. Aromatic molecules with a C=C bond are what cause the absorbance peak at 1477cm^{-1}. Alcohol, ether, ester, carboxylic acid, and anhydride having a C-O link all contribute to the absorbance peak at 1300cm^{-1}. Amines with a C-N bond are what because the broad band at 1039 cm^{-1}[18]. Due to the existence of alkenes with a C-H bond, the absorbance peak at 999 cm^{-1}, 881 cm^{-1}, and 802 cm^{-1} is present. A reduction and capping agent, C=O, C=C, C-O, C-N, and C-H functional groups are found in Ag produced with Nigella sativa extract, according to the results.

4. **Antioxidant Activity:** Due to its potential to reduce free radicals, DPPH is a well-known technique for assessing antioxidant activity. As evidenced, the Nigella Sativa seed extract's biosynthesized Ag Nanoparticles capacity to scavenge free radicals improved as

a result of the method's increasing reliance on dosage Figure (e). The mixture's hue also changed at this point. The inhibition is calculated for samples a, b, c and d are displayed in table (2) after they are taken at various concentrations. According to the table, inhibition reduces as concentration rises. In sample (a), the inhibition is 52.46% when the concentration is 150 µl, but it drops to 46.72% when the concentration is increased to 500 µl. Sample (c) exhibits inhibition at concentrations of 150 µl and 500 µl of 52.46% and48.36%, respectively. As a result, the samples (a) and (b) exhibit a higher level of inhibition compared to that of the standard as corbic solution. It is clear from the above table2 (this that) the produced silver nanoparticles have stronger antioxidant activity than the conventional solution.

5. **Antibacterial Activity:** Figure (f) Illustrates Ag's antibacterial effect against E. coli bacteria (gram negative). This indicates that the zone of inhibition exists in the E. coli bacteria. E. coli bacteria cannot thrive because of the manufactured nanoparticle. A 1.2 cm antibacterial disc (CHLORAMPHENICOL) is positioned in the center of the medium [19]. The table below contains the results of measuring the zone of inhibition at various extract concentrations. According to the table (3), for samples a, b, c, and d, the zone of inhibition was larger at 40µl and measured at 0.9, 0.8, 0.9, and 0.9cm. The outcome demonstrates that Ag NPs create an inhibition zone and prevent the growth of gram-negative E. coli bacteria.

IV. CONCLUSIONS

An efficient, chemical-free, and ecologically friendly method for synthesizing Ag nanoparticles was found in this work utilizing Nigella sativa seed extract. Simple and inexpensive co-precipitation was used to create silver nanoparticles. Silver nanoparticles crystalline structure was identified through XRD analysis. XRD patterns were used to calculate the crystalline size. The resulting crystal structure has an average size of 25.33 nm. The UV-Visible analysis was used to determine the prominent peak's absorption and the bandgap energy, which was determined to be 2.80eV. The functional groups of the Ag nanoparticles, which serve as a reducing and capping agent, were discovered using FTIR research. The study on antioxidants shows that silver nanoparticles have strong inhibition. According to a study of antibacterial study, silver nanoparticles inhibit the growth of the E.coli bacteria.

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- **Figure and Table Captions**
- **Figure (a):** Synthesis of silver oxide nanoparticles

- **Figure (b):** UV-VIS spectrum of synthesized silver nanoparticles using different volumes of extracts
- **Figure(c):** The X-ray diffraction pattern of silver nanoparticles
- **Figure (d):** FTIR analysis of Silver nanoparticles
- **Figure (e):** Antioxidant activity of Agnanoparticles
- **Figure (f):** Antibacterial activity of Agnanoparticles in *E.coli* bacteria
- **Table (1):** Structural Parameters of Silver nanoparticles
- **Table (2):** Inhibition of Agnanoparticles various concentration
- **Table (3):** Zone of Inhibition of Agnanoparticles in *E.coli* bacteria

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