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Research Article

Biofabrication and Characterization of Silver Nanoparticles using biomolecules of *Cassia fistula* Flower

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Abstract

In the last few years, the application of biological agents, like plant extracts, for the biological synthesis of nanoparticles (NPs) has become increasingly popular. This research focuses on the biosynthesis of silver NPs with the help of extract from flower of *Cassia fistula* (Cf). The study investigated several parameters, including AgNPs concentration (ranging from 1 to 100 mM), aqueous extract (100 to 900 µL), pH (4 to 10), incubation time (15 to 119 min), and temperature (30°C- 90°C) to determine their effect on the synthesis of AgNPs. The optimal conditions for synthesis were found to be a 100 mM concentration of $AgCl_2$, a temperature of 30 °C, a pH of 6.0, and duration of 120 min. The NPs were characterized by UV-Visible spectroscopy, which indicated an absorption peak at around 462 nm. X-ray diffraction (XRD) revealed that the synthesized nanoparticles showed a crystalline structure with a size extending from 78 to 120 nm, and SEM analysis demonstrated that they were nearly spherical in shape with no agglomeration.

Keywords: AgNPs; SEM; XRD; FTIR; Flower extract

1. Introduction

During the last decades, there has been an escalation of interest in nanoparticles which have demonstrated their efficacy in delivering therapeutic agents (Zhang et al., 2008). Scientists have created many diagnostic and therapeutic agents utilizing nanoparticles to treat various ailments including diabetes, allergies, and asthma (Kawasaki and Player, 2005) Green nanotechnology has gained popularity due to its low cost, eco-friendliness and scalability. Researchers have turned to plants as a favored source of materials due to their bio-reducing and stabilizing properties which makes them an eco-friendly choice (Kumar and Yadav, 2009).

In current times, there has been an increase in the study of potential uses of silver nanoparticles (AgNPs) in several areas including nonlinear optics, solar energy absorption coatings, biolabeling and catalysts for chemical reactions, and also for their antibacterial, antifungal, anti-inflammatory, anti-viral and anti-angiogenesis properties (Zargar et al., 2014; Wiley et al., 2006; Medina-Ramirez et al., 2009; Panáček et al., 2009; Nadworny et al., 2009; Remya et al., 2015). To produce AgNPs, researchers have utilized various biotic sources like fungi, bacteria, algae and plants. Among these approaches, the plant-mediated method is preferred because of its cost-effectiveness, speed, and the availability of bio-active materials like amino acids, flavonoids, terpenoids, alkaloids, and some phenolic intermediates (Gong et al., 2007; Anderson et al., 1998; Haverkamp et al., 2007). This process is linked to the idea of phytoremediation, and AgNPs generated from plants have been shown to be effective against viruses, bacteria and other eukaryotic microorganisms.

Cassia fistula (Cf), commonly referred to as Indian laburnum or Golden Shower, is a plant that is native of India. Its flowers have been used in indigenous medicine for hundreds of years to treat numerous ailments like fever, abdominal pain, skin diseases and leprosy (Perry and Metzger, 1980). Scientific studies have demonstrated that the extract derived from the flowers of C. fistula exhibits potent antibacterial and antifungal properties (Duraipandiyan and Ignacimuthu, 2007) as well as significant antioxidant activity (Bhalodia et al., 2011). Furthermore, it has been identified as having potential antidiabetic effects (Manonmani et al., 2005).

The aim of this research is to describe a simple way to green synthesized silver nanoparticle using an extract of the Cf flower at ambient temperature, no additional chemicals were added to enhance the anisotropic growth. For the characterization of the AgNPs various techniques viz., SEM- EDS, XRD, FTIR and UV- Vis Spectroscopy were used. A feasible mechanism of basic formation process of the NPs was suggested on the basis of SEM images.

2. Materials and Methods

2.1 Materials required

The Silver Chloride ($AgCl_2$) and other reagents utilized for phytochemical screening were procured from Sigma Aldrich. Tarsons Products Pvt. Ltd., India. All solutions were made in sterile Milli-Q water.

2.2. Preparation of *C. fistula* flower Extract (CF-FE)

The flowers of C. fistula were collected from the BBAU campus in Lucknow, Uttar Pradesh, India. After washing them twice with distilled water, they were air-dried for three days and crushed into a fine powder using a mortar and pestle. To create the extract of the flower, 10 g of the flower powder was mixed with 100 mL of Milli-Q water (100 mg/mL) in 500 ml beaker. The solution was heated to 75-95 ºC for an hour with stirring and kept aside to cool upto ± 25ºC. Subsequently, the flower extract (FE) was obtained by centrifuging the mixture at 7000 rpm for 15 minutes, and the remaining powder was

separated and stored at 4°C for later application. The resulting powder is referred to as the flower extract.

2.3. Synthesis of AgNPs with biomolecules of flower Extract

The procedure for synthesizing AgNPs involved the addition of 10 mL of Cf-FE aqueous solution (100 mg/ml) to a solution of 0.1 M $AgCl_2$ in 90 mL of water. The obtained mixture was then heated at 95°C for 30 minutes and stirred continuously. After this the mixture was centrifuged at 10,000 rpm for 10 minutes to get the AgNPs precipitate. The precipitate was washed twice with double distilled water and then centrifuged again at 10,000 rpm for 10 minutes. The mass was then collected, oven dried at 30-43°C, and stored for further use.

In order to optimize the reaction conditions for AgNPs synthesis, various experiments were conducted to manipulate parameters such as time, temperature, and precursor concentration. Specifically, different experiments were performed with varied incubation times ranging from 20 to 120 minutes, as well as incubation temperatures ranging from 25 to 95°C. Additionally, the concentrations of $AgCl_2$ and Cf-FE extract varied in different experiments with the concentration of $AgCl_2$ ranging from 0.25 to 0.1 mM and the extract concentration varying between 1 and 10 ml.

2.4 Characterization of Synthesized AgNPs

2.4.1 Electron Microscopy Analysis

The shape and size of the AgNPs were examined with the help of scanning electron microscope (SEM) that was combined with an energy-dispersive X-ray spectrophotometer (EDS) from JEOL, Japan (model JSM-6490LV). To prepare the samples for SEM analysis, the AgNPs were initially oven dried at 40°C for 10 hours, and then mounted onto aluminum studs with carbon tape before being platinum coated.

2.4.2 XRD Analysis

In order to assess the crystalline structure of the synthesized AgNPs, X-ray diffraction (XRD) was carried out. The AgNPs were oven dried at 40°C for 10 hours and after that they were utilized for analysis. A D8 Advance Eco XRD machine, manufactured by Bruker in Germany, was employed for this purpose.

2.4.3 FTIR Spectroscopy

Fourier transform infrared spectroscopy (FTIR) was employed to evaluate the IR spectrum of the synthesized silver nanoparticles. The FTIR characterization was executed with the help of Thermo Scientific Nicolet 6700 instrument. In order to prepare the sample for FTIR analysis, a mixture of the sample and solid potassium bromide (KBr) was utilized to create thin, transparent pellets. The pellets were then compressed using a hydraulic press. Following this, IR absorbance of the samples was measured within the wavelength range of 4000 to 400 cm^{-1}.

3. Results and Discussion

3.1 UV–Visible Spectroscopic Analysis of AgNPs

The solution incubated in the dark did not exhibit any color change even after 2 hours whereas, the solution exposed to sunlight turned darkish brown, suggesting the synthesis of AgNPs. To confirm the synthesis of AgNPs, the color change of the sunlight-exposed sample from transparent to dark brown was analyzed using a UV-Visible spectrophotometer within the spectral range of 300-700 nm. The analysis showed a distinct peak at 462 nm (refer to Fig. 1), which suggests the synthesis of AgNPs through the Surface Plasmon Resonance (SPR) phenomenon (Verma et al., 2016; Kumar et al., 2016). It should be noted that the colour change resulting from the SPR phenomenon is influenced by various factors including the size, concentration and reducing material of the nanoparticles (Kumar et al., 2016; Lee et al., 2016).

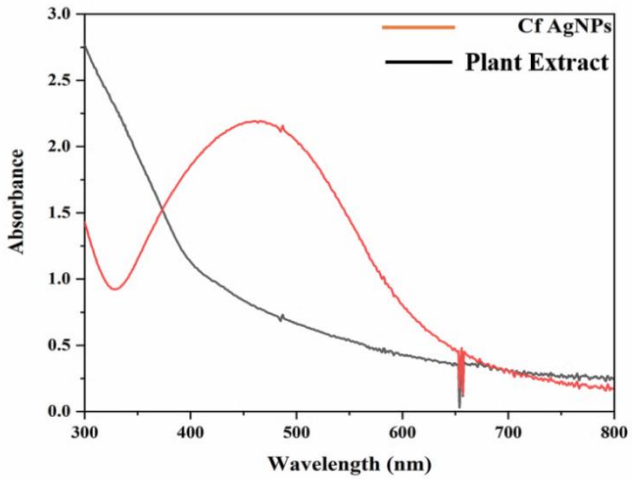
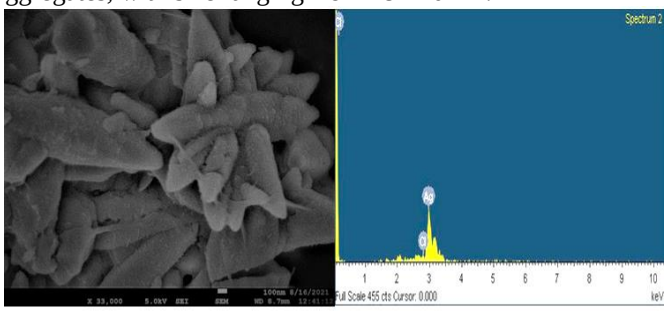


Fig. 1 Detection of biosynthesized AgNPs using extract of Cf Flower by UV–visible spectrophotometer

3.2 SEM/EDX Analysis of AgNPs

To determine the size and shape of the synthesized AgNPs, SEM analysis was employed. The obtained images of SEM disclosed the existence of both individual particles and aggregates, with size ranging from 78-120 nm.



Element	Weight%	Atomic%
Cl K	1.90	5.27
Ag L	98.10	94.73
Totals	100.00	

Fig. 2 SEM and EDX analysis of synthesized AgNPs

The majority of the individual particles had a spherical shape with an uneven surface, measuring 120 nm in size (refer to Fig. 2). The AgNPs exhibited stability and appeared to be in close proximity to each other. Some aggregated nanoparticles were also observed, characterized by slightly larger and less defined structures. EDX analysis displayed a significant peak near 3.0 keV, corresponding to Ag's binding energy, indicating the presence of high-purity Ag in the synthesized NPs (see Fig. 2).

Chlorine was also detected in the EDX analysis. The weight % of Ag was found to be 98.20, thus confirming the presence of AgNPs in the sample. Comparable findings have been reported for AgNPs synthesized from various sources, which further supports the presence of Ag (Francis et al., 2017; Dhand et al., 2016; Bocate et al., 2019).

3.3 FTIR Analysis

The functional groups present in the synthesized AgNPs were recognized by FTIR study, and the results are presented in Fig. 3. The peak perceived at 3387.0 cm^{-1} showed stretching vibrations of N-H and O-H, while the peak shift from 2921 to 2916 cm^{-1} represented stretching vibrations of C-H (Tran et al., 2013). A prominent peak at 1610.0 cm^{-1} indicated extending vibrations of C=O, as mentioned in (Kumar et al., 2019; Neethu et al., 2018). The shift of the peak ranging from 1248 to 1233 cm^{-1} signified the existence of an amide III band (Song et al., 2009). The peak at 1383.2 cm^{-1} denoted stretching vibrations of –COOH, while the absorption band at 1069.2 cm^{-1} indicates the bending vibrations of C-OH (Narayanan et al., 2010). The absorption band at 635.8 cm^{-1} indicates the bending vibrations of SO_2. The presence of functional groups like N-H, C=O and COOH may have played a vital role in the bio-reduction of Ag^+ to AgNPs, as reported by (Saravanakumar et al., 2017). Thus, the data of FTIR proved the existence of proteins as it might have been accountable for the bio-reduction of metal ions and nanoparticle formation (Suman et al., 2014). Overall, the FTIR analysis provided valuable information on the presence of functional groups in the formed Ag nanoparticles and their potential role in the bio-reduction process.

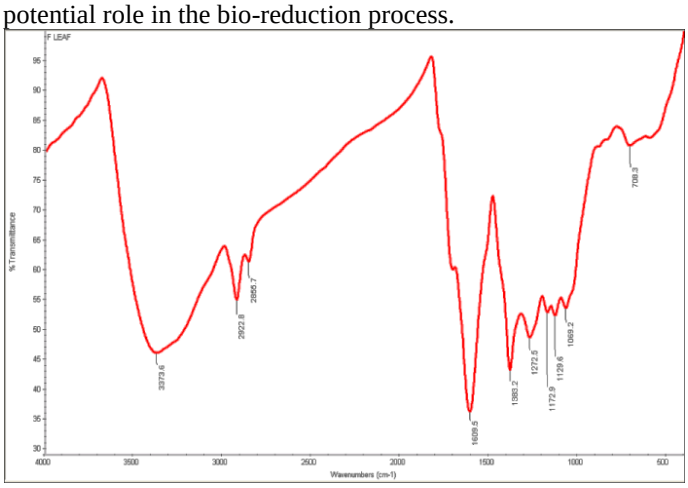


Fig. 3 Detection of biosynthesized AgNPs using extract of Cf Flower by FTIR spectra.

3.4 XRD Analysis

The crystalline structure of AgNPs synthesized from Cf flower extract was tested through XRD analysis, as illustrated in Figure 4. The XRD pattern of Cf-AgNPs showed the presence of five distinct peaks at 27.50°, 31.93° (200), 37.84° (111), 44.06° (220), and 64.25° (Fig. 3).

Additionally, two prominent peaks were observed at 2θ positions of 32.29° and 37.10°, corresponding to the (111) and (200) planes, respectively. These findings are consistent with previous research by (Wojnicki et al., 2019; Yang et al., 2016; Rajaram et al., 2015).

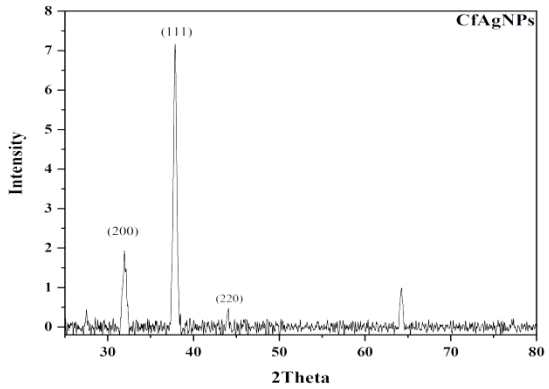


Fig. 4 Detection of biosynthesized AgNPs using extract of Cf Flower by XRD analysis.

These results provide compelling evidence that the synthesized AgNPs possess a crystalline structure. Recent studies have also reported similar findings and arrived at the same conclusion (Wojnicki et al., 2019; Yang et al., 2016; Anandalakshmi et al., 2016).

4. Conclusion

In this research we have developed easy and biological method with an average size ranging from 78-120 nm using the broth of Cf flower as reducing and capping agent. There is no use of any chemical reagent and surfactant, as a result of which this process has the advantage of being environmentally friendly. According to the results and this research AgNPs were formed by the crystallization of NPs in a linear aggregation. Furthermore, an important role was played by reaction temperature in this study and a lowered temperature favoured the formation of AgNPs. After calcification at 400°C for 2- 3 hour, the shape and size of AgNPs were hard to maintain the size.

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Author contributions

Ashvani Kumar Chaudhari (Research scholar) has conceptualize, prepared a draft of the article and formal analysis. Professor S.K. Dwivedi (Supervisor) has reviewed edited and validated manuscript.

Conflicts of Interest

There are no conflicts of interest declared by the authors

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