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Research article

XRD analysis of nanoparticles synthesized using aqueous and alcoholic extracts of *Cuscuta reflexa*

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Abstract: Plants are one of the most abundant sources of biomolecules on the planet. Researchers and academics have become more interested in the antibacterial and therapeutic characteristics of plants over the last decade. The introduction and combination of nanotechnology and phytochemistry brought up new possibilities. The synthesis of metallic nanoparticles mediated by plant extracts developed quickly and has been extensively researched. In this paper, we describe the manufacture of silver nanoparticles from *Cuscuta reflexa* aqueous and alcoholic extracts. For characterisation, the nanoparticles were subjected to X-ray diffraction (XRD) examination. XRD analysis offers concrete information about the structure and crystalline size of nanoparticles, and it can play an important role in nanoparticle characterisation. The observation includes findings for matched phases, search-match, selection criteria, peak list, crystallite size estimation, crystallinity analysis, diffraction pattern graphics, and so on. The nano size of the crystals was disclosed by XRD examination, which proved to be 38.55 nm and 66.27 nm in the case of nanoparticles synthesised using aqueous and alcoholic extracts of *Cuscuta reflexa*, respectively. The crystal system was reported to be cubic with side length of 0.4 nm, the calculated density of silver in nanoparticles was calculated to be 10.506 g/cm3 which corresponds with the density of silver element, i.e., 10.49 g/cm3.

Keywords: nanoparticles; XRD; lattice, phytochemical, crystal system.

1. Introduction

The biology of nanoparticles generated from plant extracts and other biological agents is new area of nanotechnology known as nanobiotechnology. The concept of dealing with substances at the atomic and molecular size underpins a broad field of research known as nanobiotechnology [1]. Using plant extract and other biological agents to make metallic nanoparticles is more ecologically friendly way than other technologies that are more labor-intensive, complicated, and risky [2]. On December 29, 1959, physicist Richard Feynman gave a discussion titled "There's plenty of room at the bottom" at an American Physical society meeting at California Institute of Technology, which launched the notion of nanoscience and, more specifically, nanotechnology. His presentation focused on how scientists would be able to manage and control individual atoms and molecules. Professor Norio Taniguchi created the term nanotechnology later in his research [3-4].

The notion of synthesis of metallic nanoparticles using plant extracts (polar or non-polar extraction media) obtained from plant components such as leaves, roots, fruit peals, flowers, and whole plant, etc. has gained popularity in the previous several decades. This concept gained traction and was extensively explored by authors and researchers across

the globe [1, 5-7]. Several metals, including silver [2, 8-9], zinc [10], copper [11], iron [12], and others, can be used to create plant-mediated nanoparticles. Scanning electron microscopy (SEM), UV-Visible spectrophotometer (UV-Vis), Fourier Transform Infrared (FTIR), Dynamic light scattering (DLS), and Zeta potential analysis are then used to characterize the nanoparticles for properties such as size, shape, and stability (1-2, 8-9, 11-14].

X-ray diffraction (XRD) analysis of nanoparticles (synthesized using plant extracts) is a relatively recent use of the method. The structure and crystalline size of synthesized nanoparticles are analyzed using XRD [15-17]. In this article we report the characterization of nanoparticles synthesized using aqueous and alcoholic extracts of *Cuscuta reflexa* extracts.

1. Materials and Methods

1.1. Preparation of plant powder and extract

Fresh *Cuscuta reflexa* stems were taken from Bougainvillea (host) in the Morhabadi region of India's Jharkhand state's Ranchi district. The samples were washed with deionized water, disinfected for 5 minutes with a 0.1% $HgCl_2$ solution, and dried in the shade away from direct sunlight for 45 days. Using an electrical grinder, the dry material was ground into powder. *Cuscuta reflexa* aqueous and alcoholic extracts were prepared using the Soxhlet extraction apparatus. For extraction, 50 g of the fine powder was wrapped in filter paper and placed in the thimble. Distilled water and ethanol were used separately continuously for 72 hours for aqueous and alcoholic extraction, respectively. The extracts were concentrated using a rotary flash evaporator at 45° C after filtration. The extracts were stored at room temperature in sealed containers for future use [18-19].

1.2. Phytochemical screening

The extracts were tested for phytochemical components following previously reported methods [17-21].

1.3. Synthesis of silver nanoparticles

1 ml of *Cuscuta reflexa* aqueous and alcoholic stem extracts were added separately to 99 ml of a 1mM aqueous solution of $AgNO_3$ (169.8 mg) in an Erlenmeyer flask. A magnetic stirrer was used to agitate the mixture for 4 hours continuously at 90° Celsius. The colour of the combination evolved from pale yellow to dark brown. The colour change ceased after around 2-3 hours, but the mixture was continuously stirred for up to 4 hours to eliminate the possibility of any non-reacted components. The colour change from brilliant yellow to dark brown, according reports, signifies the formation of silver nanoparticles [11 – 14]. The mixture was centrifuged at 10000 to 15000 RPM (REMI, C24 plus, India) after cooling. The supernatant was discarded to obtain a black powder. The resultant black powder was washed three times with distilled water [11-14; 17-21].

1.4. XRD analysis of synthesized nanoparticles

The nanoparticles were examined using the Rigaku-Smart Lab-X-Ray diffractometer, and the data was analysed with the XRD data processing software Match! The version 3.10.2.173 is 32-bit.

Figure 1. Details of version of Origin 9 used to analyze the data obtained from XRD analysis of nanoparticles synthesized using aqueous and alcoholic extract of *Cuscuta reflexa*

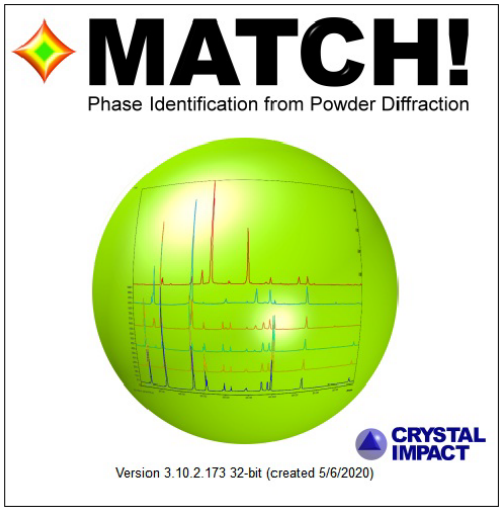

Figure 2. Details of version of MATCH! software used to analyze the data obtained from XRD analysis of nanoparticles synthesized using aqueous and alcoholic extract of *Cuscuta reflexa*

2. Results

The result of phytochemical screening of the aqueous and alcoholic extracts of *Cuscuta reflexa* is graphically presented in figure 3. The results obtained exhibited the presence of flavonoid (3.03 ± 0.04 mg/ml)), phenols (8.03 ± 0.36 mg/ml), terpenoids (5.32 ± 0.41 mg/ml) and tannins (7.52 ± 0.27 mg/ml) in the aqueous extract of *Cuscuta reflexa*. The alcoholic extract exhibited the presence of alkaloid (12 ± 1.02 mg/ml), flavonoid (3.5 ± 0.19 mg/ml), phenols (16.32 ± 0.46 mg/ml), terpenoids (17.33 ± 0.52 mg/ml) and tannins (18.35 ± 1.33 mg/ml). While flavonoids were least abundant in both the aqueous and alcoholic extracts of *Cuscuta reflexa*, phenols and tannins were found at the highest concentrations in both.

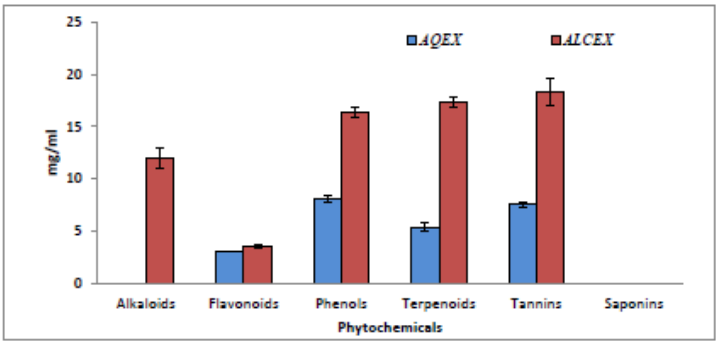

Figure 3. Phytohcemical analysis of aqueous and alcoholic extract of *Cuscuta reflexa* (mean ± SD, n=3)

Figure 4 and figure 5 shows the colour change from yellow to brown during the synthesis of silver nanoparticles using aqueous and alcoholic extracts of *Cuscuta reflexa* respectively. Figure 6 and Figure 7 shows graphical result of XRD analysis of silver nanoparticles obtained using aqueous and alcoholic extract of *Cuscuta reflexa*.

Figure 4. Colour change from yellow to brown due to reduction of $AgNO_3$ by aqueous extract of *Cuscuta reflexa*

Figure 5. Colour change from yellow to brown due to reduction of $AgNO_3$ by alcoholic extract of *Cuscuta reflexa*

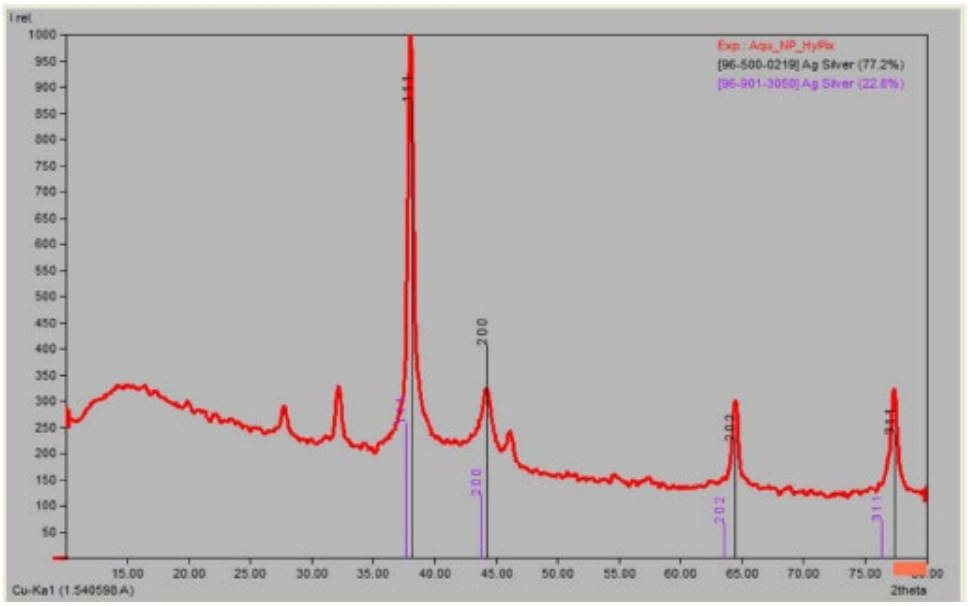

Figure 6. result of XRD analysis of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*

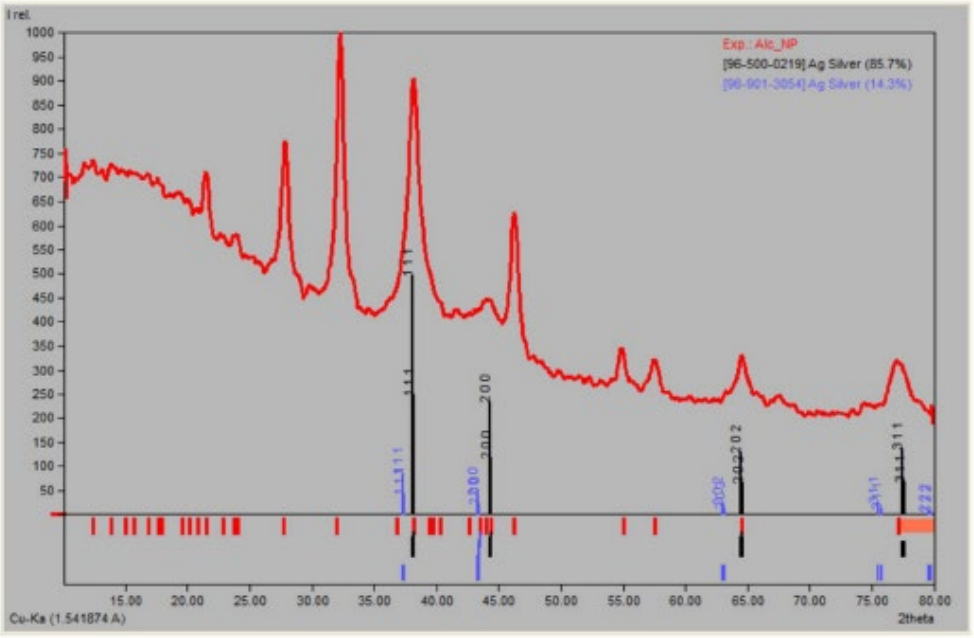

Figure 7. result of XRD analysis of silver nanoparticles synthesized using alcoholic extract of *Cuscuta reflexa*

3. Discussion

Figure 6 and figure 7 depicts the results of XRD examination of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*. The XRD examination produced a plethora of data, which were analyzed using the Match! and Origin 9 software packages. Match! software provides a platform for comparing collected data to the Joint Committee on Powder Diffraction Standards (JCPDS) database. JCPDS has been renamed the International Centre for Diffraction Data (ICDD).

The Rigaku XRD equipment was used for the study, and the parameters used were - Cu-K of wavelength 1.450598. Four main peaks were found, which corresponded to the Miller plane indices standards of Ag (22-25). This substantiates the presence of silver in nanoparticles.

The great interest in particles of nano size that has existed in the last few decades, is, to a considerable extent, due to the fact that with the decrease in size, various new properties and characteristics which are beneficial are reflected by the particles. Thus there is a common interest of workers to determine the size of the structural units. Various properties are depended on the decreasing size of particles thus, it is of great importance to primarily determine the size of particles and check whether the particle is in nano range (> 100 nm) (25).

The use of X-ray diffraction technique to determine size of particles is not new but its implementation in determining the size of silver nanoparticles synthesized using any plant extract is a new idea which has been exploited here.

XRD has a good potential for the analysis of nano-structures, because the width and shape of reflections yield information about the substructure of the materials (sizes of microcrystallites, lattice structure).

There are several approaches to analysis of X-ray diffraction line profiles, with Scherrer, Williamson-Hall, and Warren-Averbach methods being most widely applied.

Here Scherrer method has been utilized to estimate the crystallite size. X-ray is an electromagnetic radiation produced by bombarding high energy electron on any heavy metal. According to Mosley's law every element has its unique signature in the form of Kα emission. An electromagnetic radiation can be diffracted by atomic planes only when half of its wavelength is less than inter-atomic distance (D), i.e.

$$\lambda / 2 \leq d$$

Here

λ is wavelength of X-ray

d is inter-atomic distance

Nowadays monochromators are used in which an X-ray source is used to emit CuKα radiation only. Therefore the wavelength (λ) = 0.154 nm is used for investigation of samples.

In an X-ray diffractometer there is a source of X-ray emission, one sample holder on which the X-ray falls and the diffracted rays are detected by the detectors. In this system the sample remains stationary and the source and detector both moves. The peaks are the typical XRD data in which variation of angle i.e. 2θ is shown in x axis and the intensity is shown in y axis. At some particular position there are some peaks, their intensity is not the same and also these peaks are not absolutely sharp, these peaks have some width.

The position, intensity and width of these peaks are analyzed. For crystallite size estimation the size of coherently diffracted piece of crystal can be calculated using Scherrer's equation.

$$D = \frac{K\lambda}{BCos\theta}$$

Here

D is diameter of crystallite

K is Scherrer's constant (generally taken as 0.9)

B is FWHM (full width half maxima) of the peak

θ is half of the corresponding angle

The result of XRD analysis of silver nanoparticles synthesized using aqueous and alcoholic extract of *Cuscuta reflexa* are expressed as peaks obtained from XRD data (figure 6 and figure 7). The peaks are compared with Miller's plane of indices. 2θ is shown in X-axis and intensity is shown in Y-axis. The peaks obtained by XRD analysis nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* coincided with 111, 200, 202 and 311 plane of Miller's indices of Ag [96-500-0219] and Ag [96-901-3050]. The wavelength of X-ray (CuKα) emission was 1.540598 Å. The data range was 10.000° – 80.000°.

The observation contains results under several heads such as Matched Phases, Search-Match, Selection Criteria, Peak list, Crystallite size estimation using Scherrer Formula, Degree of crystallinity analysis, Integrated profile areas, Peak residuals, Diffraction pattern graphics.

From the obtained report the crystallite size in case of silver nanoparticles synthesized using aqueous and alcoholic extract of *Cuscuta reflexa* was estimated to be 38.55 nm and 66.27 nm respectively. The Scherrer constant used in calculation was 0.94. The crystal system was reported to be cubic with side length of 0.4 nm. The calculated density of silver in the nanoparticles was calculated to be 10.506 g/cm3 which corresponds with the density of silver element i.e. 10.49g/cm3. From the results it is clear that the particles were in nano range and it was further confirmed that silver atom is responsible for formation of nanoparticles (25, 26).

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