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Biosynthesis and characterization of silver nanoparticles by using green tea leaf extract from *Camellia sinensis*

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Abstract
Green synthesis of silver nanoparticles (AGNPS) is a rapid developing field in Nanotechnology. In this study, we have investigated the AgNPs were biosynthesized from aqueous silver nitrate through a simple and eco-friendly route using *Camellia sinensis* leaf extracts. Which has a rich source of polyphenolic compounds used for the reduction and capping of silver nanoparticles. The synthesis was carried out for 2 h at room temperature, and the reduction process of Ag^{+} to Ag^{0} was observed by the change of color from clear brown to milky grey. Characterization of the silver nanoparticles was performed by using UV-Vis, TEM, FT-IR and XRD. The UV-Vis spectrum of the aqueous medium containing silver nanoparticles showed an absorption peak at around 425 - 435 nm. Well-dispersed silver nanoparticles with an approximate size of 4 nm were observed in the TEM image. FT-IR spectral analysis indicated the tea extract showed the reducing and capping agents on the surface of AgNPs. The XRD analysis shows that the synthesized AgNPs are of face-centered cubic structure. In conclusion of our study, AgNPs plays a major role in the field of nanotechnology and nanomedicine.

Keywords: silver nanoparticles, camellia sinensis, green synthesis, UV-Vis, TEM, FT-IR and XRD

Introduction
Green nanotechnology is an area with significant focus at present on the important objective of facilitating the manufacture of nanotechnology-based products that are eco-friendly and safer for all beings, with sustainable commercial viability [1]. The development of toxicity free metal nanoparticles has become a great challenge in recent times. The primary challenge in this focal area is the maximization of the usage of environmental friendly materials in the generation of metal nanoparticles [2, 3]. Metal nanoparticles, especially the noble metals, have mainly been studied because of their strong optical absorption in the visible region caused by the collective excitation of free-electron gas [4].

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level [5]. Due to its small size; it produces structures, devices, and systems with atleast one novel/superior characteristic property. These properties may differ in important ways from the properties of bulk materials and single atoms and molecules [6]. The chemical synthesis of nanoparticles may lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in its application. The synthesis of nanoparticles by green method can potentially eliminate this problem. Therefore, there is an urgent need to develop a green process of nanoparticle synthesis. Green synthesis methods employing either biological microorganisms or plant extracts have emerged as a simple and alternative to chemical synthesis. Green synthesis provides advancements over chemical methods as it is environment friendly, cost effective, and easily scaled up for large scale synthesis. Generally, the green synthesis method involves three main steps, (1) solvent medium selection, (2) environmental benign reducing agent selection, and (3) non-toxic substances for nanoparticles stability selection [7]. The synthesis of nanoparticles by using plant extracts can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can be suitably scaled up for large scale production under non-aseptic environments.

Silver nanoparticles are well known as one of the most universal antimicrobial substances in the field of biology and medicine due to their strong biocidal effect against microbial species, which has been used for centuries to prevent and treat various diseases, most notably infections [8]. In addition, tea leaf extract was used for the AgNPs synthesis. Begum *et al*. reported the AgNPs synthesized by the ethyl acetate extract of tea leaves [9]. Nadagouda *et al*. showed the synthesized AgNPs with the size range of 20–60 nm [10]. However, the reaction conditions, including the temperature or tea extract dosage, the synthesis mechanism, the AgNPs stability, and the antibacterial activity have not been fully investigated.

Previous studies showed that AgNPs would likely release silver ions after entering the aquatic environment [11, 12] which would reduce the stability of AgNPs. In addition, silver ions exhibited different physiochemical properties and biological toxicity from AgNPs [12, 13, 14]. Silver nanoparticles also reported to possess anti-fungal [15] anti-inflammatory [16] anti-viral [17]. Recently, the development of silver nanoparticles is expanding. They are now used as part of clothing, food containers, wound dressings, ointments, and implant coatings. Some silver nanoparticles' applications have received approval from the US Food and Drug Administration [18]. In the present work, we have proposed a green chemical method for synthesizing AgNPs using Green tea leaves extract from Camellia sinensis as reducing and capping agents. To the best of our knowledge, this study is new and presents a simple methodology to synthesize

AgNPs efficiently at room temperature.

Materials and Methods

Plant material

The plant material C*amellia sinensis* were collected from Tata tea estate, Munnar, Kerala. The leaves are naturally shade dried and powdered by using kitchen blender.

Synthesis of Silver nanoparticles

The Green tea leaves extract was prepared by weighing 10 g of Green tea leaves in 500 mL beaker along with 100 mL of distilled water and maintained at 60°C for 10 min before decanting it. The solution was filtered by 0.45 µm Millipore membrane filter and followed by 0.2 µm Millipore membrane filter. For synthesis of silver nanoparticles, 100 mL of AgNO3 (1 mM) was reacted with 12 mL of the tea extract in Erlenmeyer flask at room temperature. Color changes of the solution were observed.

Characterization

The crystalline and phases of the Ag nanoparticles were characterized by X-ray diffractometer (XRD-6000, Shimadzu, Japan) with Cukα radiation (λ = 1.5412 Å) in the range of 10°–80° with 2°/min scanning rate. The functional and composition of Ag nanoparticles were characterized by Fourier-Transform Infrared (FTIR, Perkin Elmer, Spectrum BX) spectroscopy in the range 4000–280 cm-1. In addition, the optical property of prepared Ag nanoparticles was analyzed via UV-visible (UV-Vis, Perkin Elmer, Lambda 35) absorption double beam spectrophotometer with a deuterium and tungsten iodine lamp in the range from 300–600 nm at room temperature.

The morphology of the prepared Ag nanoparticles was observed by Transmission Electron Microscopy (TEM, Hitachi, and H7100). Ag nanoparticles were sonicated for 15 min by a sonicator (50 Hz, Soniclean). Then, the dispersed solution was dipped to a copper grid at room temperature. After drying, sample was analyzed at 80 kV. The particle size distributions were determined using UTHSCSA Image Tool Program (version 3.00; Dental Diagnostic Science, UTHSCSA, San Antonio, TX).

Results and Discussion

The color change was noted by visual observation in the Erlenmeyer flask which contains AgNO3 solution with green tea extract. The color of the AgNO3/tea extract solution changed from colorless to light brown after 2 hour and eventually to milky grey. (Figure 1).

Fig 1: Aqueous solution of AgNO3 with green tea leaves extract (A) before adding the tea extract and (B) after addition of tea extract at 2 hour

This color change indicates the formation of Ag nanoparticles in the solution. Tea extract without AgNO3 did not show any color changes. The formation of Ag nanoparticles was further confirmed by using UV-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier-Transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM).

Figure 2 shows the UV-Vis absorption spectrum of the synthesized Ag nanoparticles. Silver nanoparticles have free electrons, which give surface Plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of silver nanoparticles in resonance with light wave. A broad absorption peak was observed at 425 – 435 nm, which is a characteristic band for the Ag. No other peak was observed in the spectrum which confirms that the synthesized products are Ag only.

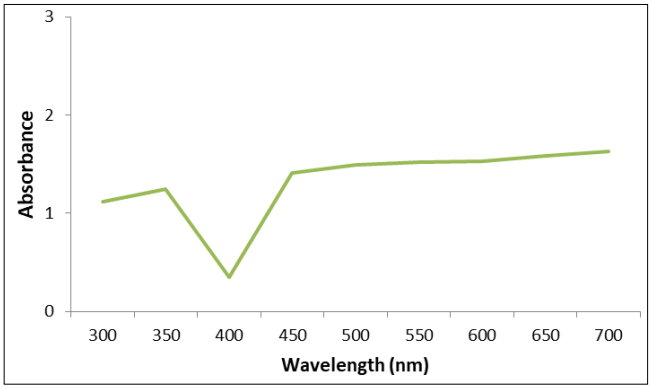

Fig 2: UV-Vis spectrum of AgNPs

Figure 3 shows the synthesized AgNPs was confirmed by the characteristic peaks observed in the XRD image. All diffraction diffraction lines observed at 2θ angle 37.59°, 44.29°, 64.68°, and 76.98° respectively, have been indexed as (111), (200), (220) and (311) respectively. XRD patterns were analyzed to determine peak intensity, position and width, full-width at half-maximum (FWHM) data was used with the Scherer formula explained in section materials and method. The average particle size of AgNPs can be calculated using the Debye–Scherer equation: where K is the Scherer constant with value from 0.9 to 1 (shape factor), where λ is the X-ray wavelength (1.5418 Å), β½ is the width of the XRD peak at half-height and θ is the Bragg angle. From the Scherer equation, the average crystallite size of Ag-NPs for the sample at 24 hours. The typical XRD pattern revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles.

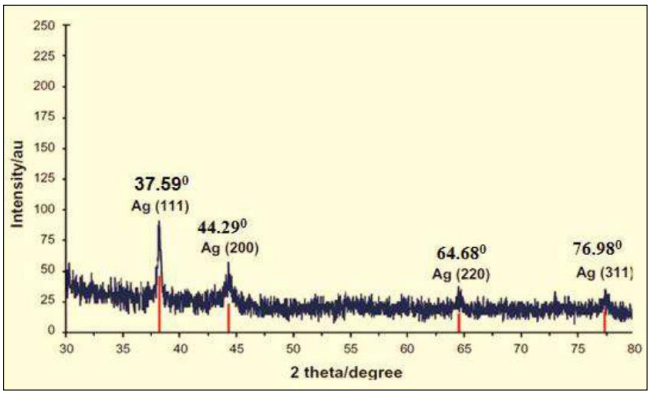

Fig 3: XRD patterns of AgNPs

FTIR measurement was carried out to identify the possible bimolecular responsible for capping and reducing agent for the Ag nanoparticles synthesized by tea leaf extract. Three obvious infrared bands are observed at 3313 cm^{-1}, 2912 cm^{-1} and 1701 cm^{-1} (Figure 4). The intense broad band at 1635 cm^{-1} is due to N−H and O−H stretching mode in the linkage of the proteins. The medium intense band at 1018 cm^{-1} arises from the C = O stretching mode in amine I group which is commonly found in the protein [22] indicating the presence of proteins as capping agent for silver nanoparticles which increases the stability of the nanoparticles synthesized. On the other hand, the intense and broad peak at 3313 cm^{-1} corresponded to the Ag metal.

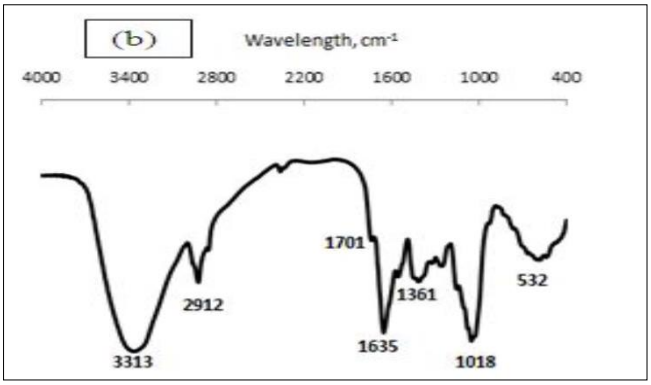

Fig 4: FT-IR spectrum of AgNPs.

Transmission electron microscopy (TEM) has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles. The TEM image of silver nanoparticles is shown in Figure 5. From the image, it is evident that the morphology of silver nanoparticles is spherical which is in agreement with the shape of SPR band in the UV-vis spectrum. Figure 5B shows the histogram of size distribution of silver nanoparticles. The average particles size measured from the TEM image is 4.06 nm which is in good agreement with the particle size calculated from XRD analysis.

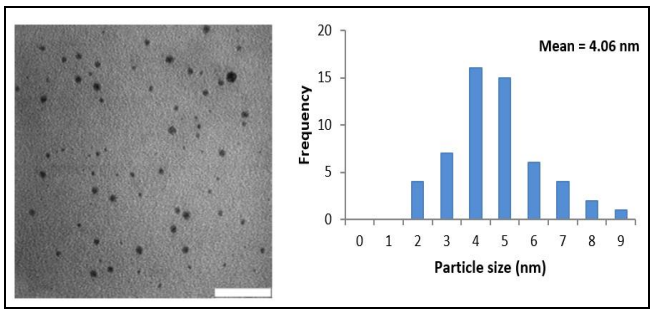

Fig 5: (A) Tem image (B) Particles size distribution of AgNPs synthesized by C. sinensis.

The environmentally benign silver nanoparticles synthesis process has potential applications in various fields. Silver nanoparticles can be applied in coating for solar energy, bio-labeling, food packaging, antimicrobial agent and drug delivery [19, 20] besides that, silver nanoparticles play an important role in the medical area. Silver nanoparticles act as biomarker in detection of early diagnosis and therapy monitoring such as the detection of tumor for cancer treatment and early diagnosis for Alzheimer's disease [21].

Conclusion

In this paper, we report a green approach for the synthesis of Ag nanoparticles using green tea leaves extract. This is a simple, green and efficient method to synthesize silver nanoparticles at room temperature without using any harmful reducing agents such as n-hexane and any capping or dispersing agent. It was concluded that the green synthesized silver nanoparticles were composed of spherical particles which were highly crystalline. The particles sizes were controlled in the range from 2 to 10 nm.

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