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LOW COST AND RAPID BIOSYNTHESIS OF SILVER NANOPARTICLES USING *CASSIA TORA* LEAF EXTRACT

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ABSTRACT: Chemical, physical, and biological methods have been developed to synthesis nanoparticles but chemical and physical methods are involved in the production of toxic by-products which are hazardous moreover the methods are very expensive. To synthesis stable metal nanoparticles with controlled size and shape, there have been searched for inexpensive, safe, and reliable and "green" approach. The present study reports an environmentally friendly, low cost, novel and rapid method for synthesis of silver nanoparticles. We have developed a green synthetic method for silver nanoparticles using *Cassia tora* leaf extract which acts as a reducing & capping agent. It was observed that use of *Cassia tora* leaf extract makes rapid and convenient method for synthesis of silver nanoparticles and can reduce silver ions into silver nanoparticles within few seconds of reaction time. UV-Vis spectrometer uses to monitor the reduction of Ag ions and formation of AgNPs in medium. The morphological studies of the biosynthesized nanoparticles are done using UV-Vis, HRTEM & FESEM techniques FTIR was performed to identify the functional groups of carbonyl, hydroxyl, amine and protein molecule which form a layer covering AgNPs and stabilize the AgNPs in medium.

INTRODUCTION: Green chemistry is an innovative method of research which includes application part for design and development enabling efficient production of the goods that can bring the hazardous health substances to a minimum. The green chemistry concerning bio, organic, inorganic, analytical, and even physical chemistry emphasizes only on large - scale applications. These new green nanotechnology projects aim at minimizing the human as well as the environmental health hazards [1].

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The recent vibrant development of nanotechnology-based drug delivery is creating the smart and novel approach; the functionalized noble nanoparticles can themselves act as drugs or drug vehicles and can also be employed for many applications in pharmaceutical and medical research.

Noble metal nanoparticles are being widely used nowadays in the fields of medicine, biology, material science, physics, and chemistry [2]. Metal nanoparticles have been shown to possess enormous application potential in the areas such as photography, catalysis, biological labeling, photonics, optoelectronics and surface-enhanced Raman scattering (SERS). Traditionally nano-particles have been prepared and stabilized by several physical and chemical methods; of them, chemical reduction, electrochemical techniques and photochemical reduction are being most widely used. Of the noble metal nanoparticles, gold and silver are of particular interest because of distinctive properties, such as good electrical conductivity, chemical stability, catalytic and antibacterial activity [3]. Most of the traditionally used chemical and physical methods of synthesis of metal nanoparticles are expensive and often involve the use of toxic, hazardous chemicals which may pose environmental risks.

Also, most of the current synthetic methods mostly rely on the use of organic solvents due to the hydrophobicity of the capping agents used. Lately the quest for cleaner methods of synthesis has led to the development of bio-inspired approaches. Bio-inspired methods have been put forward to be advantageous over other synthetic methods as they are cost-effective and do not involve the use of toxic chemicals, high pressure, energy, and temperatures [4]. Recently a number of organisms have been used for the synthesis of nanoparticles, noble metals in particular. Synthesis using bio-organisms is compatible with the green chemistry principles: the bio-organism is eco-friendly as are the reducing agent employed and the capping agent of the reaction. Microbe mediated synthesis of nanoparticles is not industrially feasible as they require the maintenance of highly aseptic conditions.

Compared to microbe assisted synthesis, plant-mediated synthesis of nanoparticles is a relatively under-exploited field and is recently gaining wide attention [5]. The inorganic metal nanoparticles can fight against human pathogens and dangerous diseases like cancer. Silver has been widely used since ancient times to fight infections and control spoilage. *Cassia tora* found to have significant anti-metastatic effect on cancer cells as demonstrated by decreased mRNA expression of matrix metalloprotease (MMP) [6]. Keeping this in view, we have synthesized silver nanoparticles for their possible applications as anti-cancer and anti-inflammatory agent using *Cassia tora* leaf extract by green synthesis method. Silver nanoparticles of *Cassia tora* have been characterized spectroscopically and microscopically.

MATERIALS AND METHODS: Fresh leaves of *Cassia tora* were collected from the Botanical garden of Dr. Y. S. Parmar University. The botanical identity of the plant specimen of *Cassia tora* was confirmed by Dr. M. Khan taxonomist at Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, India. It was authenticated (no. - Tech/Herb(Ident.)/13-14/Acc No. 108502) to be *Cassia tora* leaf belonging to the *Fabaceae* family. Analytical grade $AgNO_3$ was procured from Hi-media Labs.

Biosynthesis of Silver Nanoparticles Using *Cassia tora* Leaf Extract: Analytical grade $AgNO_3$ prepared in 10^{-3} M concentration was used for the experiment. Fresh plant leaf (10 gm) cut into small size was taken in a wide neck borosil flask and washed thoroughly using double distilled water. Then 200 ml of double distilled water is poured into the flask and subjected to microwave heating for 3 min to subdue the enzymes and proteins which interferes the reduction process. The solution is then filtered in hot condition using 10 µm meshes to remove the solid fibrous residues.

The clear filtrate (extracellular extract) was used for nanoparticles synthesis [7, 8, 9, 10]. Silver nitrate solution was interacted with the plant extract (1:1) mixing ratio for different time periods at 30°C in a rotary shaker at 120 rpm. Immediately after the addition plant extract to $AgNO_3$ aqueous solution, a light yellowish color was observed which changed to dark brown color. This change of color indicates the formation of silver nanoparticles.

Characterization:

UV-Visible Spectroscopy: UV-visible spectroscopy is a simple and quite a sensitive technique that can be used to detect the formation of silver nanoparticles [11, 12]. The reduction of silver ions to the nanoparticle form was monitored by measuring the UV-visible spectra of the solutions after diluting the sample with millipore water 20 times. The spectra were recorded on UV-visible double beam spectrophotometer from 200 to 700 nm.

X-Ray Diffraction: The purified powders obtained after 4 h of interaction under laboratory conditions was subjected to X-ray diffraction analysis. The generator was operated at 40 kV and with a 30 mA current. The scanning range was selected between 10 and 100^{θ} angles.

Particle Size Analysis: The particle size range of the nanoparticles along with its polydispersity was determined using SALD-7500 particle size analyzer. Particle size was arrived based on measuring the time-dependent fluctuation of scattering of laser light by the nanoparticles undergoing Brownian motion.

HR-TEM Analysis: The characterization of nanoparticles was carried out by High-Resolution Transmission Electron Microscopy (HRTEM) using lyophilized samples. TEM samples of the metal nanoparticles synthesized were prepared by placing drops of the product solution onto carbon-coated copper grids. Grid was completely dried and examined by TEM at 200 kV.

FESEM-EDS: Silver nanoparticles were examined by FESEM equipped with an energy dispersive spectrometer (EDS). Analyzed samples were dried at room conditions for 5 days and small fragments were placed on pin stubs and then coated with carbon under vacuum.

RESULTS AND DISCUSSION: The reaction mixture containing aqueous $AgNO_3$ solution and *Cassia tora* leaf extract was exposed to microwave radiation and the UV-visible spectrum **Fig. 2** of the biosynthesis. Surface plasmon peak observed confirms the influence of aqueous *Cassia tora* leaf extract in reducing Ag^+ ions to Ag nanoparticle from aqueous $AgNO_3$ solution. Absorbance intensity increases steadily as a function of reaction time and it is observed that the surface plasmon peak occurs at 450 nm. It is observed from the study that the reaction is completed in a very short time**.** The current method of biosynthesis of AgNPs has a time-related advantage over conventional and benign methods using other plants or microbial extracts as the rate of synthesis is as same as physical and chemical methods.

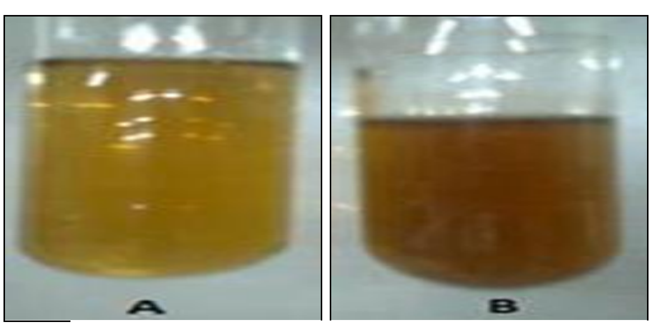

FIG. 1: (A) LEAF EXTRACT WITHOUT SILVER NITRATE. (B) LEAF EXTRACT WITH SILVER NITRATE

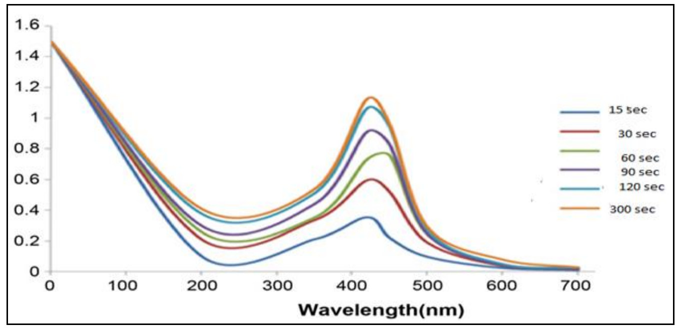

FIG. 2: UV-VIS SPECTRA INDICATING MICROWAVE-ASSISTED SILVER NANOPARTICLES SYNTHESIS RECORDED AS A FUNCTION OF TIME

XRD investigation was carried out to confirm the crystalline nature of silver nanoparticles and the result has been shown in **Fig. 3**. Prominent peaks at (111), (200), (220) and (311) are the silver nano-particles peaks and match the standard JCPDS file 04-0783 pattern.

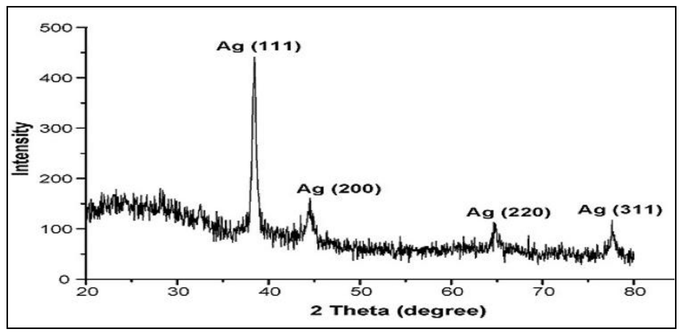

FIG. 3: XRD PATTERNS OF CRYSTALLINE SILVER NANOPARTICLES

The peak at 3421 cm^{-1} and 2923 cm^{-1} refers to NH stretch vibration of primary and secondary amides of protein. The peak at 2853 cm^{-1} refers to C-H symmetrical stretch vibration of alkanes. The peak at 2361 cm^{-1} refers to the primary amine group of protein. The peak at 1629 cm^{-1} refers to carbonyl stretch, which is assigned to the amide I bond of protein. The peaks at 1457 refer to amino and amino-methyl stretching groups of protein (Shown in **Fig. 4**).

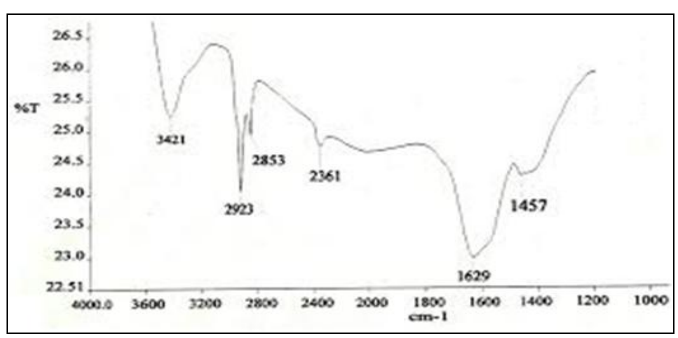

FIG. 4: FTIR ANALYSIS OF BIOSYNTHESIZE SILVER NANOPARTICLES

IR spectroscopic study confirmed that carbonyl group form amino acid residues and proteins have the stronger ability to bind metal, could possibly form a layer covering the metal nanoparticles (*i.e.*, capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium [13, 14]. These results suggest that the biological molecules perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. The proteins present over the silver nanoparticle surface may act as capping agent for stabilization.

The bio-moiety capping which also confers the stability of silver nanoparticles colloidal solution produced by this modus is noteworthy. FESEM images at different resolution are shown in **Fig. 5**. If observed carefully a layer of bio moiety is seen covering the metal surface of all nanoparticles. This organic moiety might have taken part in the reduction of Ag^+ ions to silver nanoparticles. **Fig. 6** shows TEM image which gives a clear understanding of the morphology of functionalized silver nanoparticles. The particles are nearly spherical in shape having 23 ± 2 nm sizes.

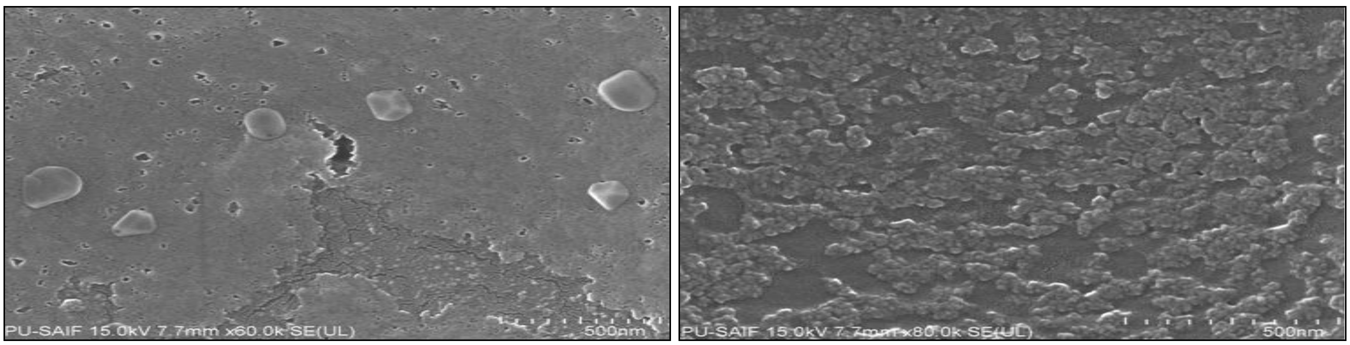

FIG. 5: FESEM IMAGES OF SILVER NANOPARTICLES

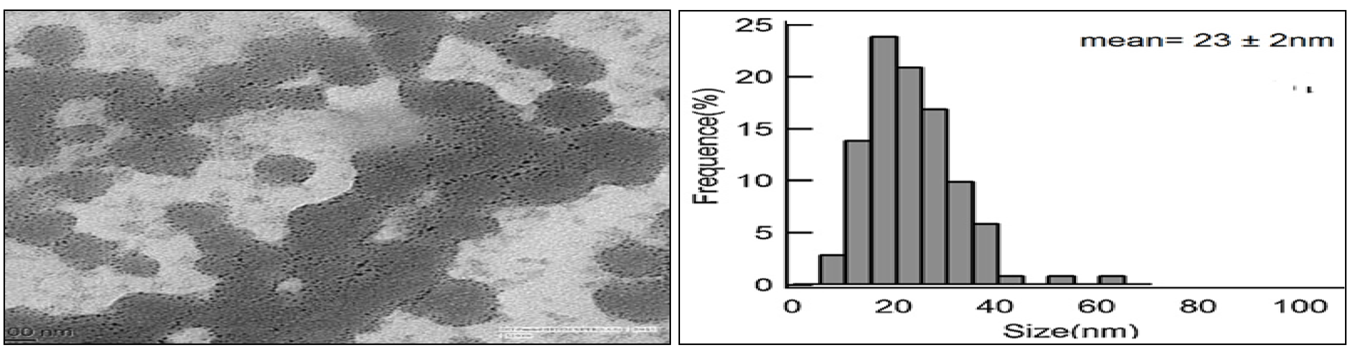

FIG. 6: (A) HR-TEM IMAGE OF SILVER NANOPARTICLES AND (B) SIZE DISTRIBUTION HISTOGRAM SILVER NANOPARTICLES

CONCLUSION: *Cassia tora* leaf extracts have been used as reducing and capping agent for the synthesis of silver nanoparticles. Since this plant is easily grown and easily available all over India. *Cassia tora* leaf extracts based silver nanoparticles have been synthesized through a green chemistry approach. This is a simple, green and rapid method to synthesize silver nanoparticles at room temperature without using any harmful reducing agents.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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