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Short Communication

Green synthesis of silver nanoparticles using acacia concinna plant extract and their antibacterial activity

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Abstract

Rapid synthesis of AgNPs by plant of Acacia concinna fruit extract. Synthesis of Ag Nps has been planned as a valuable and eco-friendly approach. This research work are cost effective as well as eco-friendly i.e reduction method using the fruit extract of acacia concinna plant and aqueous silver nitrate solution, for the preparation of the AgNPs. This plant extract act as both reducing and capping agent. The essential ingredients liable variables are triterpenes, flavonoids and eugenol, are present in the fruit extract that used for the formation of AgNPs. After the reduction process silver (Ag^+) ions was monitored, it showed the formation of Ag NPs using UV-visible spectrophotometry, which exhibits the absorption peak around at 430 nm. The sizes of nanoparticles were calculated by transmission electron microscopy (TEM) and X-ray diffraction (XRD). In addition, by using Agar-Well Diffusion Method, the antimicrobial activity of synthesized AgNPs was investigated by against Escherichia coli. For the conversion of Ag^+ to AgNPs at room temperature, this method could be proved a better substitute to chemical synthesis method without the contribution of any hazardous to environment.

Keywords: Eco friendly, TEM, UV-visible, XRD, diffusion method etc.

Introduction

Recently, synthesis of noble metal NPs such as Au, Ag, Pd and Pt and their study are given much importance[1-3]. Their physical as well as chemical properties are look like different from the bulk materials which fascinate the current research field[4]. Noble metal nanoparticles are of much significance owing to their optical, electronic and magnetic as well as catalytic behavior. Mainly PXRD technique is used to study the phase and purity of nanomaterials. In this research work, we discussed about the easy, low cost and room temperature synthesis of metal AgNPs. The as prepared AgNPs are of 2-20 nm size and their respective results are exhibited by TEM analysis. To prepare nanoparticles on solid support materials, including diverse lithographic techniques, vacuum depositionl, diffusion, electrophoretic chemical and electrochemical deposition of the metal NPs etc.[5-8] a range of synthetic techniques has been adopted. For consistent morphology and size of NPs, there are a variety of techniques like vacuum deposition of metal, diffusion, lithography etc and but they all are not cost-effective. The appropriate, simplest and economical approaches to synthesize nanomaterials are using the green and environmentally benign method.

Currently, the green synthetic method has gained fabulous persuade in large-scale biosynthesis process than physical and chemical process due to environmental concerns. The green synthesis of NPs is a clean, nontoxic, nonhazardous, cost-effective and environmentally friendly approach. The biological synthesis process includes use of the plant (or) and microbes (fungi, bacteria, algae) for the synthesis. Due to adverse microbial culture protection the use of plant extract is more significant than using microbes, time-consuming and cost-effective. In this research work, we have confirmed the synthesis of AgNPs via green synthesis method using *Acacia Concinna* plant extract. We have also investigated using UV-Vis spectroscopy, ATR, biological activity against Gram (-) ve (Escherichia coli (E. coli) bacteria, PXRD data. The PXRD results were correlated with TEM results.

Materials and methods

Silver nitrates (99.99%) were purchased from sigma Aldrich and *Acacia concinna* plant fruit extract as a green reducing agent and all valuable glassware's were cleaned with distilled water during the experiment.

Aqueous extract of Acacia concinna fruit: Acacia concinna plant fruit were collected from University of Rajasthan, Jaipur, Rajasthan, and cleaned with distilled water and dry at normal room temperature and grind to make it powder. 2 gm of *Acacia concinna* fruit powder was dissolved with 50 mL of distilled water and kept for 12 h, afterwards the extracts were filtered by whatman paper and stored at 4°C for further nanoparticles synthesis process.

Synthesis of silver nanoparticle: Firstly 10 ml of *Acacia concinna* plant fruit extract and freshly 10 mM silver nitrate solution prepared in a separate beaker. Secondly, silver nitrate solution was stirred for half an hour and afterwards the plant fruit extract was drops wise added to aqueous $AgNO_3$. The titration was carried out for reducing of Ag^+ ions to Ag nanoparticles. The obtain solution is dissolved for on 15 minutes and then incubated at near room temperature for 24 h. The change in color was observed in the solution that confirms the formation of the NPs in the solution.

Figure 1: Acacia concinna fruit.

Separation of AgNPs: The synthesized Ag NPs was separated by centrifugation technique at 4000 rpm for 30 min. For further settlement of particles the supernatant material was transferred to a beaker and frequent centrifugation process was carried out to clean AgNPs. The obtained synthesized pellet was dry in an oven and stored for further characterizations.

Characterization: The absorption spectra of samples were observed at 200-800 nm wavelengths using by UV-visible spectrophotometer (LAB India, UV 3000^+). Using FTIR spectrum, the synthesized NPs were analyzed for the existence of biomolecules (Thermo Scientific Nicolet 380 FTIR Spectrometer). In transmission mode at the frequency range between 4000 and 400 cm^{-1} with the resolution of 4 cm^{-1}, the IR spectra of the synthesized nanoparticles were recorded by using ATR spectrophotometer (T-alpha, Bruker) for liquid sample. The nature of Ag nanoparticles is crystalline as evaluated by X-ray diffraction method. The synthesized Ag nanoparticles study on size, shape and the size distribution of nanoparticles of colloidal solution were analyzed by transmission electron microscopy (JEOL JEM2100 TEM).

Estimation of antibacterial activity: The antibacterial activities of synthesized Ag NPs, from the relevant plant extracts were successfully against Gram (-) ve (Escherichia coli (E. coli) bacteria for investigation. The microorganisms use in the study are purchase from the market. Disc diffusion method[9] was featured for trying of plant extract and their synthesized respective Ag NPs solution. The discs were soaking with distilled water, plant extract, pure $AgNO_3$ solution and solution containing AgNPs of each type individually. Then the discs were air dry at room temperature. The plates containing nutrient agar media were ready by swabbing them with the microbial cultures. The discs were located in the subsequent manner, disc soaked with distilled water as negative control, disc soaked with plant extract, disc soaked with 1 mM silver nitrate solution and disc soaked with solution of synthesized AgNPs. The dishes were incubated at 37°C for 24 to 48 h. Then, the maximum zone of inhibition was experimental and calculated for analysis beside of microorganism.

Results and discussion

Effect of reaction time on the formation of AgNPs, by visual observation was noted, when Acacia concinna fruit extract is added to aqueous silver salt ($AgNO_3$), colour will be changed from pale yellow to yellowish brown and lastly show the brown colour, as shown in Figure-2. By the reduction of silver salt, the optically observed and vary in colour of the solution indicates the creation of metal silver nanoparticles.

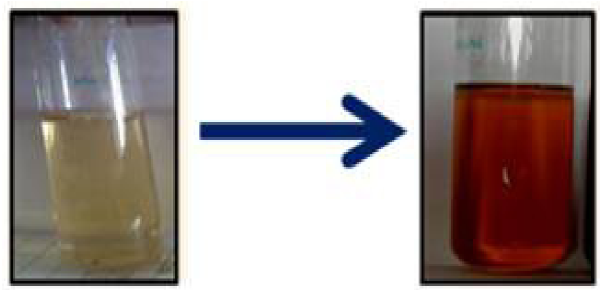

Figure-2: Shows the coloration due to formation of Ag NPs.

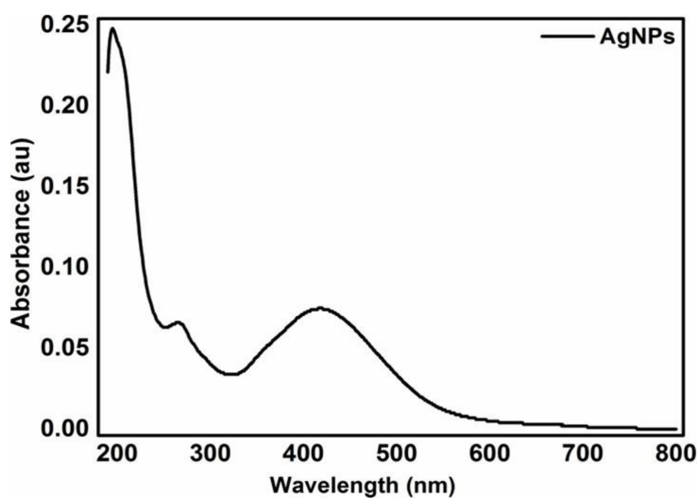

Figure-3: UV-visible spectra of the synthesized Ag NPs from predicted optimum conditions upon dilution of 3.5 times. Inset Figure shows the coloration due to formation of Ag NPs.

Synthesized AgNPs show the yellowish brown colour from pale yellow solution due to the excitation of (SPR) surface plasmon resonance. The change in color was appeared initially after 3 min. of adding the salt solution to the acacia concinna fruit broth. After 24 h, the colour of the solution becomes stable, which indicates that no silver salt was missing in solution for further reaction. In the present study, the synthesized nanoparticles are spherical in shape as suggested by SPR band, which is further confirmed by TEM study. Then the synthesized silver nanoparticles was examined and showed a broad adsorption peak around at 430 nm, which is liable for the Ag[10] and confirmed by respective absorption spectra. The synthesized AgNPs are uniform size distribution and absorption maximum is practically observed at around 430 nm. Due to the strong surface plasmon resonance (SPR) the UV absorption spectrum appeared, i.e., Silver nanoparticles responsible for surface Plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of synthesized AgNPs in resonance with light wave. The resulted absorption spectra are size dependent and SPR band depends on the refractive index of the solution. Figure-3 spectra of the absorption due to the synthesis of AgNPs which is appeared from the reaction of acacia concinna fruit extract with $AgNO_3$ solution recorded in between the range of 200-800 nm.

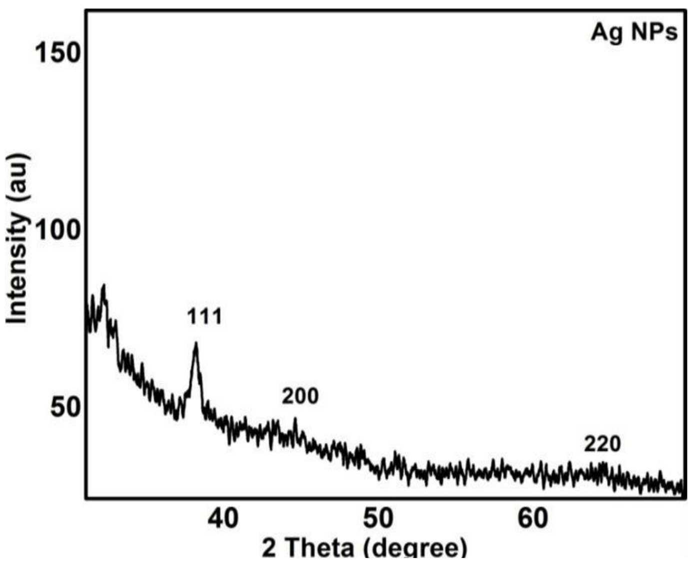

Figure-4: Shows XRD patterns for Ag nanoparticles.

Figure-4 Acacia concinna fruit extract broth containing AgNPs were centrifuged at 20,000 rpm for 20 min and to get rid of any unwanted impurities, the sterile distilled water was used for during this process. The purified pellet was then dried at 60°C at 12h and the sample was characterized by the X-ray diffraction (XRD) using a Bruker D2 Phase diffractometer that was operated in transmission mode with CuKα radiation (λ = 1.5418 Å). At the interval of 0.02° with a counting time of 30 s per step, the data were collected over the 2θ range from 20° to 90°. Figure-4 shows XRD results for synthesized Ag nanoparticles by pure acacia concinna fruit extract. Three major distinguishing diffraction peaks for AgNPs were experimental at 2θ = 38.4, 44.5, 64.8 which correspond to the (111), (200) and (220) crystallographic planes of face-centered cubic Ag crystals, respectively (JCPDS 00-004-0783) with a lattice parameter of a = 4.077 Å. In general, the crystallite size of Ag NPs is related to width of XRD peaks and Debye-Scherrer equation used for determines average crystallite size. To calculate crystalline size the (111) plane was chosen. The average particle size of synthesized AgNPs is found to be 3.42 nm from Debye-Scherrer equation. This result was consistent with the TEM study.

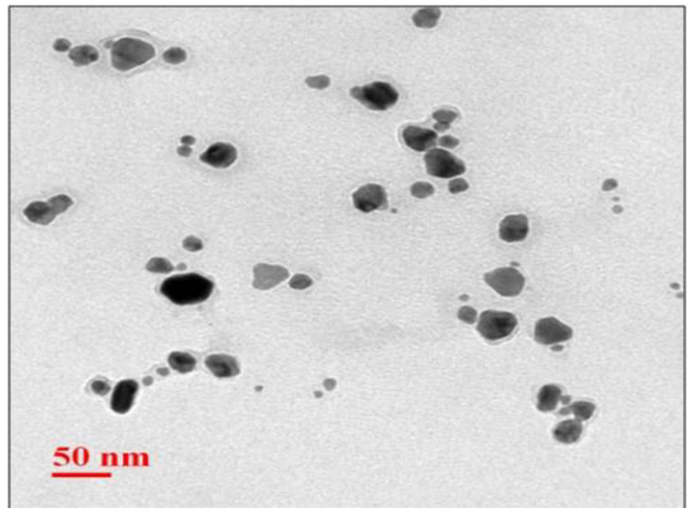

Figure-5: TEM images of synthesized AgNPs.

In Figure-5 TEM image of the Ag NPs corresponds to the topography, which particle size exhibits in between 2-20 nm and suggested the presence of approximately spherical size of silver nanoparticles. The image indicates the dispersity and morphology of the Ag NPs. These particles are distributed in smaller and bigger size zones and rounded in shape and size. These synthesized Ag NPs may be due to the capping agent as well as phytochemicals such as polyphenols or due to the formation of the cluster.

The present biomolecules are liable for capping agent as well as proficient stabilization of the AgNPs by ATR investigation. The IR spectrum of synthesized AgNPs shows the intense powerful bands around at 3315.79 and 1637.36 cm^{-1} are identified for O-H stretching, H-bond and C=C stretch respectively. The IR spectrum of plant extract has exposed in Figure-6. The IR bands located at 3315.79 cm^{-1} and 1634.37 cm^{-1}, clearly show significant variance among the spectral positions of IR bands due to the reduction process. The analysis of IR band peaks confirmed the absorbed alkaloids, phenols as well as terpenoids on the surface of AgNPs[11].

The IR results also provided and show an idea with reference to biomolecules approach at different functionalities. The probable mechanism designed for the reduction of Ag^+ to AgNPs be able of explained as: The present phenolic OH groups are in hydrolysable, and tannins can form transitional complexes with suitable Ag^+ ions, which for that reason go through oxidation to quinone forms. This results in the successive form of Ag^+ to Ag nanoparticles[12].

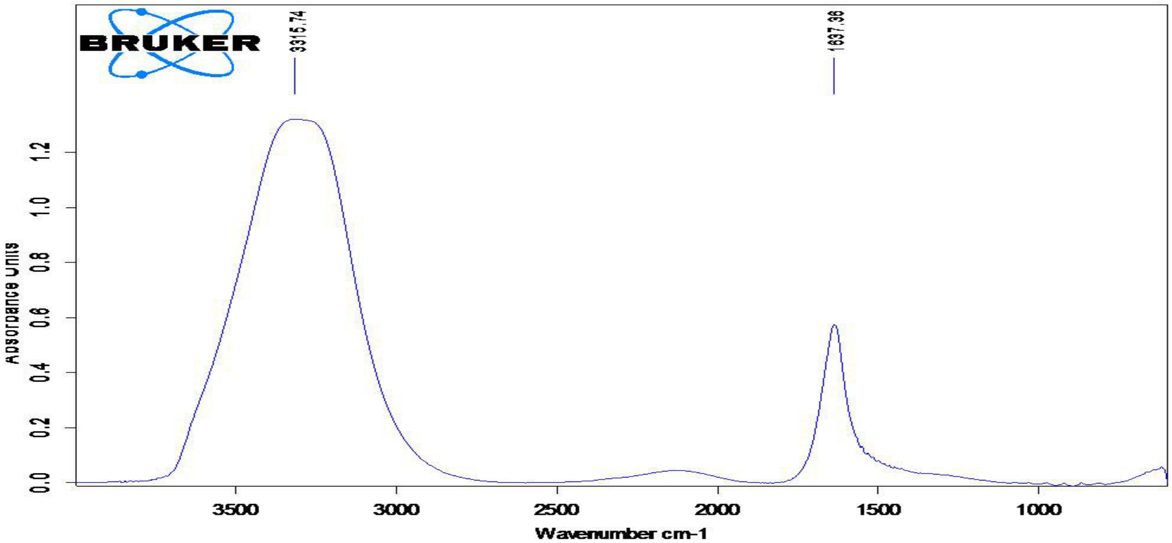

Figure-6: ATR spectrum of biosynthesized AgNPs.

Antibacterial activities: In the present research study of the antibacterial activity of the AgNPs which is analysed against E.Coli bacterial colony presented from sample is exposed in following information (Figure-7). Different quantities of synthesized AgNPs (2, 4, 8 and 10mg) are further to the agar dishes containing bacterial colony. The dishes are visible for each dilution and bacterial colony is observed results after incubating the agar plates overnight at 37^{o}C. Zone of approval is experiential maximum at 10 mg of AgNPs. The antibacterial activity results show due to modify in the bacteria cell membrane permeability as well as degradation of enzymes in bacteria by synthesized AgNPs. The result zone of inhibition increased as well as increases the concentration of AgNPs.

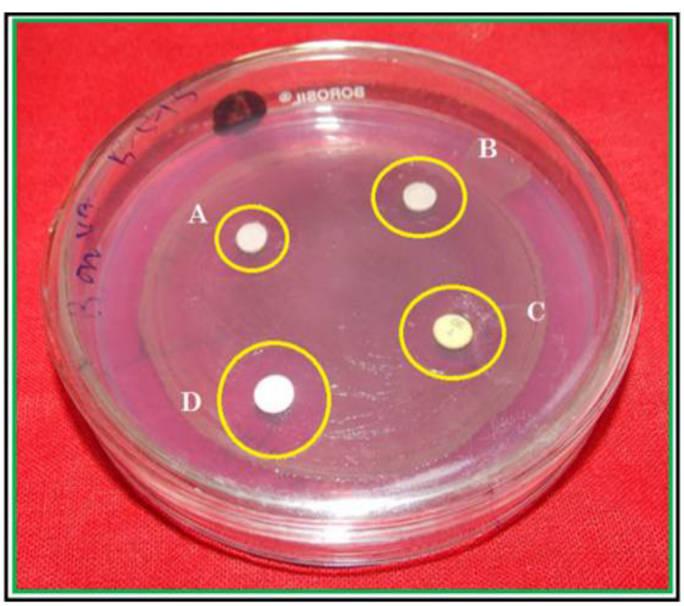

Figure-7: The inhibition zones of Ag NPs against *E.coli on nutrient agar. Where, the samples A, B, C and D are the as synthesized AgNPs in 2, 4, 8 and 10mg, respectively.*

Conclusion

In this report, we have synthesized AgNPs using Acacia Concinna plant extract via a biological method. Here the plant extract useful for reducing agent as well as stabilizing. Which are responsible for silver metal to nano-size (2-20 nm) and to stabilize the NPs. The as-synthesized AgNPs were examined using TEM, XRD, UV-Vis, and FTIR. The formation of nanoparticles, as well as size, was investigated by using UV-Vis spectroscopy. The crystal structure of the nanoparticle as well as average particle size of the NPs were investigated by PXRD analysis and further the shape and size were demonstrated by TEM analysis. This research work can be further extended to explore the synthesis and optical properties of AgNPs using other plant extracts which can be of medicinal use.

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