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Green synthesis of silver nanoparticles by using *Acacia concinna* fruit extract and their antibacterial activity

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ABSTRACT

This report briefly introduce cost effective, green and eco-friendly approach for synthesis of silver nanoparticles (AgNP's) using *Acacia concinna* fruit extract. After exposing the silver ions to the fruit extract, the rapid reduction of silver ions led to the formation of stable AgNP's in solution due to reducing and stabilizing properties of *Acacia concinna* fruit extract. The synthesized AgNP's were characterized by UV-Vis spectroscopy, it shows absorption peak at 450nm. The size and morphology of nanoparticles were analyzed by transmission electron microscopy (TEM). In addition, the antimicrobial activity of as prepared AgNP's was investigated against *Escherichia coli* using Agar Well Diffusion Method. This newly developed method for synthesis of AgNP's could prove a better substitute for the physical and chemical methods currently used.

KEYWORDS

Green synthesis;
AgNP's;
Acacia concinna fruit extract;
Characterization;
Antibacterial.

INTRODUCTION

The unique features of nanoparticles may lead to play an important role in biomedicine, energy science, optics and other health care applications[3]. Among the various nanoparticles, AgNP's are widely investigated owing to their broad range of applications as antibacterial agent, catalyst and as a biosensor. Several researches have made attempts for synthesis of AgNP's using chemical reduction[11], electrochemical reduction and photochemical reduction[22]. These methods employ harsh reducing and stabilizing agents in the synthesis process that may create some dicey effects in biomedical applications[9, 26]. To overcome such tedious methods and prevent utilization of highly toxic materials, the interest in this field has shifted towards green approach. These approaches focused on utilization of environment friendly, cost effective and biocompatible reducing agents for synthesis of AgNP's.

The green synthesis of AgNP's has attracted much attention in recent years because these synthesis protocols are low cost and more eco-friendly than standard methods of synthesis[17].Convential methods of AgNP's synthesis requires more chemicals, large quantities of energy and results in formation of hazardous byproducts[1]. The use of plant and their extacts for synthesis of AgNP's can be advantageous over other biological synthesis process. The use of plant extract reduce the cost as well as we do

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not require any special culture preparation and isolation techniques[12]. The advantages of using plants and their extracts for the synthesis of metal nanoparticle is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction[21].

Several works has been reported for synthesis of AgNP's by using microorganisms such as bacteria,[5], either intracellularly[14] and extracellularly[12,15], fungus[7] and algae[20, 19]. Recently, plant extract assisted syntheses of nanoparticle have attractive interest in the area of nanoscience and technology due to its eco-friendly nature. Nanoparticles produced by plant extracts are more stable and rate of synthesis is faster than in the case of microorganisms. Some of plant materials such as leaves[1, 16, 4] stem[23], seeds[9], fruits[25, 8], latex[10] and barks[18] are involved in metal reduction process.

In the present study, we have synthesized AgNP's using *Acacia concinna* fruit extract for reduction of Ag^+ ions to Ag^0 from silver nitrate solution. *Acacia concinna* is relatively large genus of plants belonging to *fabaceae* family. *Acacia concinna* is climbing shrub native to Asia, common in the warm plains of central and South India. It has been used traditionally for hair care in Indian subcontient. It is one of the Ayurvedic medicinal plants[29]. The fruit is known in India as *shikakai*. Further, several of organic compounds present in *Acacia concinna* extract. The Figure 1(a) shows the *Acacia concinna* shrub and Figure1(b) shows *Acacia concinna* fruit.

The synthesis of AgNP's by using *Acacia concinna* was reported. But in this reported work, a strong reducing agent $NaBH_4$ was used for synthesis of AgNP's[13]. Here in, we report the green synthesis and characterization of AgNP's generated by the reduction of *Acacia concinna* fruit extract. The synthesized AgNP's were characterized by using different characterization techniques and tested against pathogenic microorganisms.

EXPERIMENTAL

Materials

Silver nitrate ($AgNO_3$) of analytical grade was purchased from Sigma-Aldrich Chemicals and was used without further purification. *Acacia concinna* fruit was collected from *Acacia concinnna* shrub. For the preparation of fruit extract and synthesis method distilled deionized water was used.

Preparation of Acacia concinna fruit extract

The fruit was collected from *Acacia concinna*

Figure 1 : a] Acacia concinna b] Acacia concinna fruit

shrub. The fruit was dried to remove moisture and grind in the mixer. About 0.5 g of dried powder was added into some distilled water and boiled it for 15 min. After boiling, the solution was cooled and the cooled content was filtered. The yellow colored filtrate was diluted to 50ml with distilled water. This solution was used for synthesis of AgNP's.

Synthesis of AgNP's using *Acacia concinna* fruit extract

A stock solution of 4 mmol·L^{-1} $AgNO_3$ was prepared and kept against sunlight. In a typical procedure, 10 ml of fruit extract (1 %) was taken and 25 mL $AgNO_3$ stock solution was added to it with constant stirring at room temperature. The yellow color of solution changes to brown color slowly. The yellow color solution turned brown color indicated the formation of AgNP's as shown in Figure 2. This synthesis process was studied time to time by using UV-visible spectra.

Characterizations

The synthesized AgNP's were characterized by using various techniques. The nanoparticles were primarily characterized by UV Visible spectroscopy, which has proved to be a very useful technique for analysis of nanoparticles. UV Visible absorption spectram of AgNP's was recorded on (Shimadzu, Model UV-3600) UV Visible spectrophotometer. Morphology and size of AgNP's were investigated by transmission electron microscopy (TEM JEM-2100). The antimicrobial activity of bio-synthesized AgNP's were studied by using Agar Well Diffusion Method.

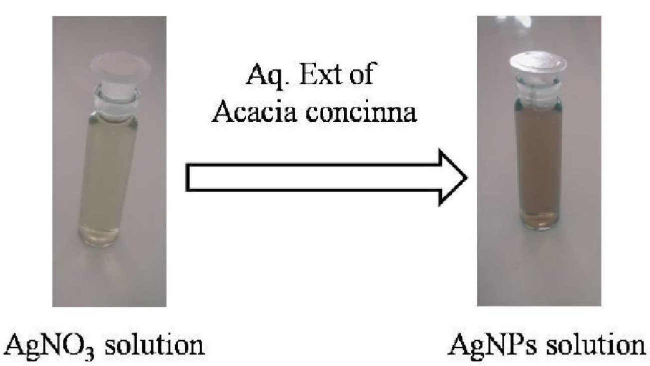

Figure 2 : Synthesized AgNP's by using *Acacia concinna* fruit extract

RESULT AND DISCUSSION

UV-Vis spectral analysis of AgNP's

The addition of silver nitrate solution to *Acacia concinna* fruit extract resulted in color change of solution from yellow to dark brown, due to the production of AgNP's. The formation of of nanoparticle was firstly characterized by UV visible spectroscopy analysis. It is well known that AgNP's exihibit dark brown color in aqueous solution due to excitation of surface plasmon resonance[27, 6, 24] and formation of dark brown color because of oscillation of free electron in the AgNP's. After 24 hours the strong SPR band was observed at 450nm and broadning of peak indicated that the particles were polydispersed. The time dependent UV-Vis absorption spectra during the progress of the formation of AgNP's are illustrated in Figure 3.

TEM studies of AgNP's

Transmission electron microscopy (TEM) was used to view the morphology and size of AgNP's. TEM grids were prepared by placing a drop of the particle solution on a carbon coated copper grid and drying under lamp. Figure 4(a) shows the typical bright field TEM micrograph of the synthesized AgNP's. It is observed that most of the AgNP's were spherical in shape. A few agglomerated AgNP's were also observed in some places, thereby indicating possible sedimentation at a later time. It is evident that there is variation in particle sizes and the average size estimated was 18 nm and the particles size ranged from 10 nm to 35 nm. As can be seen in Figure 4(b) presence of rings patterns in the selected area electron diffraction (SAED) reveals the single face centered cubic (fcc) crystalline nature of the spherical nanoparticles. The HR-TEM image of single AgNP's is presented in Figure 4(c) showing the spherical morphology of nanoparticles

Antibacterial activity

Biosynthesized AgNP's were analyzed for antimicrobial activity against *Escherichia coli* by Agar Well Diffusion Method. In this method petriplates containing 20mL Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were

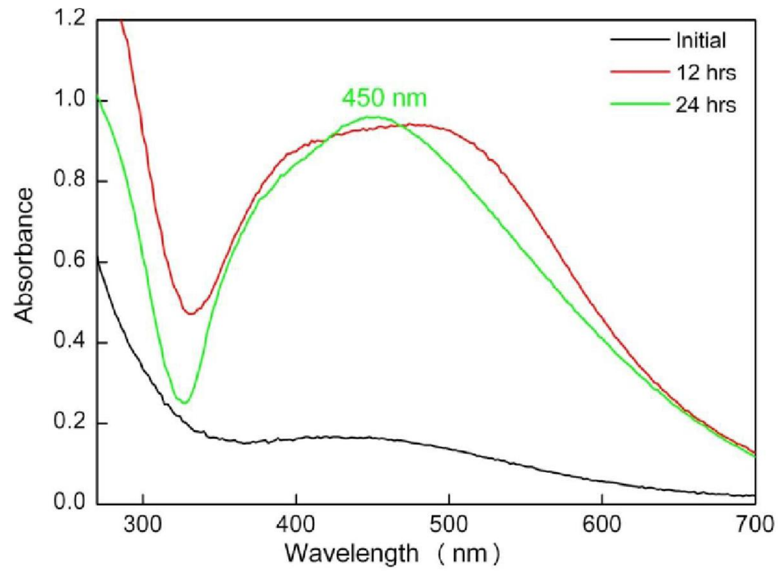

Figure 3 : Time dependent UV- absorption spectra of AgNP's

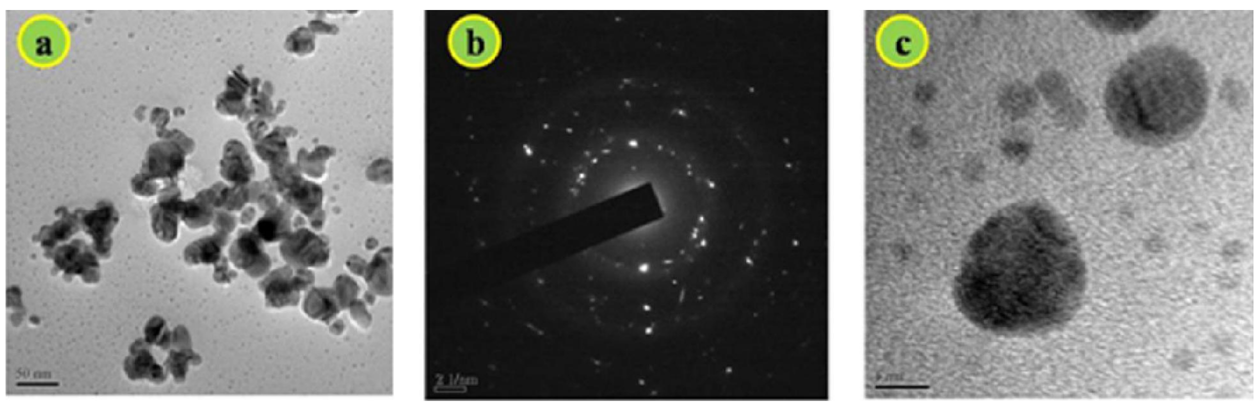

Figure 4 : a] TEM images of AgNP's b] SAED image of AgNP's c] HR-TEM image of AgNP's

TABLE 1 : Antibacterial activity of AgNP's

Test organism	Zone of inhibition (diameter in mm)		
	Acacia concinna fruit extract	AgNP's 100ppm	AgNP's 50ppm
Escherichia coli	0	16	14

cut and AgNP's were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The result indicated the synthesized AgNP's using *Acacia concinna* fruit extract have stronger antimicrobial activity. The TABLE (1) show the summarized result of antibacterial activity. The image for antibacterial activity of biosynthesized AgNP's against *Escherichia coli* is shown in Figure 5.

The mechanism of antibacterial activity of AgNP's on microorganisms is partially known. Silver particles have positive charge, it will attach with the cell wall of bacteria by electrostatic attraction and disrtupt the cell permeability and respiration due to generation of the reactive oxygen species. AgNP's bind with thiol groups of DNA and RNA which affect the protein synthesis of bacteria. The AgNP's closely associated with the cell wall of bacteria by forming 'pits'and finally it affects the permeability and cause cell death.[27]. The antimicrobial activity of silver depends on the size of particles. Smaller particles having larger surface area available for interaction and they easily penetreate into the cell will give more bactericidal effect than the larger particles[28].

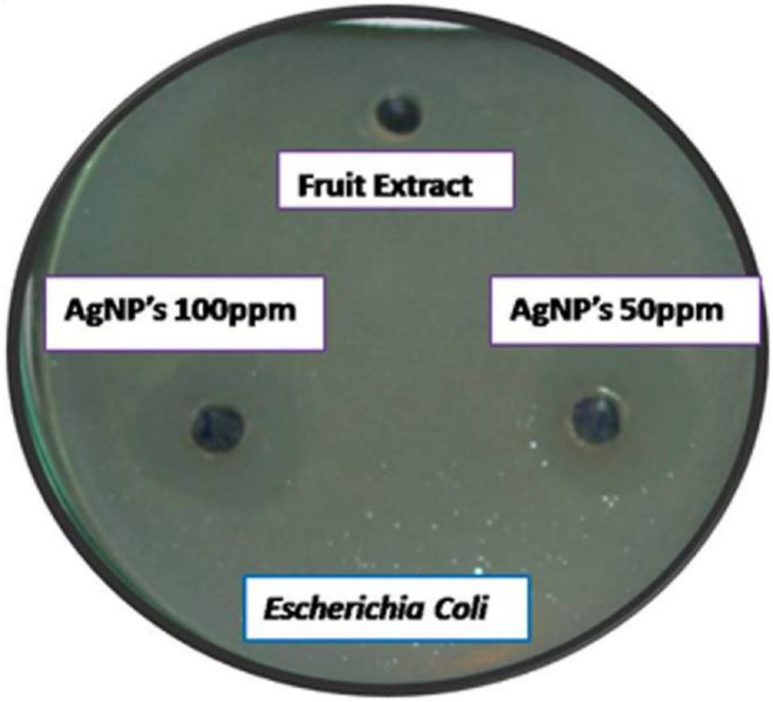

Figure 5 : Biosynthesized AgNP's against *Escherichia coli*

CONCLUSION

We have developed a fast, eco-friendly and convenient method for the synthesis of AgNP's using *Acacia concinna* fruit extract. The formation of AgNP was demonstrated by UV-visible spectroscopy in the absorbance peak at 450nm. The particle size of AgNP was in the range 10-35nm established by TEM. Green synthesized AgNP's had the bactericidal activity against *Escherichia coli* was successfully demonstrated by Agar Well Diffusion Method. Therefore, this green chemistry approach towards the green synthesis of AgNP's has many advantages such as, ease with which the process can be scaled up, economic viability etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical applications, makes this method potentially exciting for the large scale synthesis of other inorganic materials.

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