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Research Article

CESTRUM NOCTURNUM: AN EMERGING NANOFACTORY FOR THE GREEN SYNTHESIS OF SILVER AND COPPER NANOPARTICLES AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The objective of this research was to develop reliable green processes for the synthesis of multiple metallic nanoparticles from a single plant system. In this investigation, we have focused on a single plant system for the synthesis of silver, as well as copper nanoparticles from the *Cestrum nocturnum* leaf extract, which acts as a reducing and capping agent. Synthesized nanoparticles were characterized using Transmission Electron Microscopy (TEM), which revealed spherical silver nanoparticles of the size range 10 nm to 37 nm and spherical copper nanoparticles of the size range 10 nm to 30 nm. Antibacterial activity of the generated nanoparticles was evaluated against *Escherichia coli* using a well diffusion assay. Compared to silver nanoparticles, copper nanoparticles were found to be more effective as an antibacterial agent against *Escherichia coli*. Present study concluded that this method is a simple, efficient, eco-friendly and cost-effective alternative for the synthesis of silver and copper nanoparticles.

Keywords: Nanoparticles, Silver, Copper, Antibacterial, Biosynthesis, TEM

INTRODUCTION

The ever-increasing use of nanotechnology in the field of biological sciences can be accredited to their numerous unique properties[1]. Nanotechnology is the manipulation of matter on the atomic, molecular or supramolecular level, of the size 1-100 nm[2] that show properties not found in the bulk samples of the same material[3]. The study of these peculiar features has always been of great interest to scientists. Silver and copper nanoparticles have attracted significant attention because of their wide range of applications such as superconductors, sensors, catalytic, optical and electrical. Further, they can be used as an antimicrobial and anti-fungal agent[4].

Various methods of nanoparticle synthesis have been developed and classified into physical, chemical and biological. However, most of the physical and chemical techniques require large investments and various hazardous chemicals, stabilizing agents and capping agents[5,6]. Recently, biological synthesis of nanoparticles has emerged at the intersection of nanotechnology and biotechnology, and has received tremendous attention due to a growing need for the development of environmentally benign technologies for nanomaterial synthesis.

Thus, green nanotechnology has been proposed as the environmental friendly alternative to chemical and physical methods[7]. Lately, the use of microorganisms to synthesize nanoparticles has garnered much interest, but maintaining a cell culture is very difficult due to continuous sub-culturing and risk of contamination[8]. Therefore, researchers turned towards the green route due to ease of availability of plants in nature. As a result, plant systems are one of the best candidates for nanoparticle generation[9].

Nanoparticles are known to be versatile and have a wide range of applications in modern biology, medicine and bio-detection of pathogens[10]. Nanoparticles derived from biological sources have been shown to have antibacterial and antifungal properties[11,12]. Due to rising health-care costs and rise in incidents of infections and antibiotic resistance, there is an urgent need to develop new antimicrobial molecules.

The current investigation was aimed to develop a simple method for the synthesis of silver and copper nanoparticles using a single plant system, *Cestrum nocturnum* and evaluate its antibacterial activity against Gram negative bacteria *Escherichia coli*.

MATERIALS AND METHODS

Plant Materials

Plant leaves of *Cestrum nocturnum* were collected locally from Aurangabad, Maharashtra, India.

Chemicals

Silver nitrate ($AgNO_3$) was purchased from Merck Ltd., Mumbai. Copper sulphate was purchased from HiMedia. Freshly prepared double distilled water was used during the experimental work.

Microorganisms

Antibacterial assay was carried out on *E. coli* using well diffusion technique. The microbial culture was maintained by Department of Biotechnology, MGM's Institute of Biosciences & Technology.

Preparation of Plant Extract

Fresh leaves from the plant *Cestrum nocturnum* were collected, washed thoroughly with tap water, rinsed with distilled water and blot-dried. 25 gm of leaves were then cut into small pieces, grounded using a mortar pestle and homogenized with 100 ml of double-distilled water. The obtained plant extract was filtered using Whatman filter paper No. 1 and filtered extract was stored at 4ºC for further experimental use.

Synthesis of Silver Nanoparticles

10 ml filtered plant extract was added to 10 ml of 3mM $AgNO_3$. The test tube containing the reaction mixture was kept at 80ºC-90ºC for 20 minutes in a boiling water bath and then incubated at 35ºC-37ºC in an incubator for 24 hours. The reaction mixture was centrifuged at 3,000 rpm for 5 minutes and the pellet was discarded.

Synthesis of Copper Nanoparticles

10 ml filtered plant extract was added to10 ml of 3mM $CuSO_4$. The test tube containing the reaction mixture was kept at 80ºC-90ºC for 20 minutes in a boiling water bath and then incubated at 35ºC-37ºC in an incubator for 24 hours. The reaction mixture was centrifuged at 3,000 rpm for 5 minutes and the pellet was discarded.

TEM Analysis

TEM analysis was used to confirm the synthesis of nanoparticles and understand their size and shape. Prior to the sample preparation for TEM analysis, sonication of sample was done for 10 min. A thin film of the sample was prepared on a small copper grid and allowed to dry. The TEM analysis was performed on a PHILIPS- Model No- CM200 instrument at IIT-SAIF, Bombay.

Antibacterial Activity Study

Evaluation of antibacterial activity of the generated nanoparticles was performed against *E. coli* using well diffusion method. Using a cork borer, five wells were created in the LB agar. 25 µl, 50 µl, 75 µl and 100 µl of nanoparticle solution were added to the wells and plant extract was added to the central well as control. All the petri plates were incubated at 37ºC for 24 hours. After incubation, the zone of inhibition was measured.

RESULTS
Visual Observation

A noticeable colour change was observed in the reaction mixture after a period of incubation with respect to the control i.e. plant extract. This provided a primary observational confirmation of the presence of nanoparticles in the reaction mixture.

TEM Analysis

To further confirm the presence of nanoparticles and understand their size and morphology, Transmission Electron Microscopy was used.
The analysis revealed spherical nanoparticles in the case of silver nanoparticles as well as copper nanoparticles as shown in Figure 2. The size range of silver nanoparticles was found to be between 10 nm to 37 nm (Figure 2.A), whereas the size range of the copper nanoparticles was between 10 nm to 30 nm (Figure 2.B). This provided conclusive proof that leaf extract of *Cestrum nocturnum* plant can produce silver and copper nanoparticles.

Antibacterial Activity Study

The antibacterial properties of the silver and copper nanoparticles were evaluated against Gram-negative bacteria *E. coli* using well diffusion technique and the diameters of the zone of inhibition were measured (Table 1).

It was observed that silver nanoparticles generated by the leaf extract of *Cestrum nocturnum* plant showed no antibacterial activity against *E. coli* (Figure 3.A). But, copper nanoparticles synthesized by the leaf extract of the same plant showed good antibacterial activity against *E. coli* (Figure 3.B). The zone of inhibition at various concentrations of the nanoparticles solution was measured as shown in Table 1.

It was found that *E. coli* is more sensitive to copper nanoparticles as compared to silver nanoparticles; the maximum zone of inhibition for *E. coli* was measured as 25 mm at 100 µl of copper nanoparticles.

Table 1: Antibacterial activity of silver and copper nanoparticles against E. coli

Nanoparticles	Zone of inhibition (mm)				
	Control	**25 µl**	**50 µl**	**75 µl**	**100 µl**
Silver nanoparticles	-	-	-	-	-
Copper nanoparticles	-	18	20	22	25

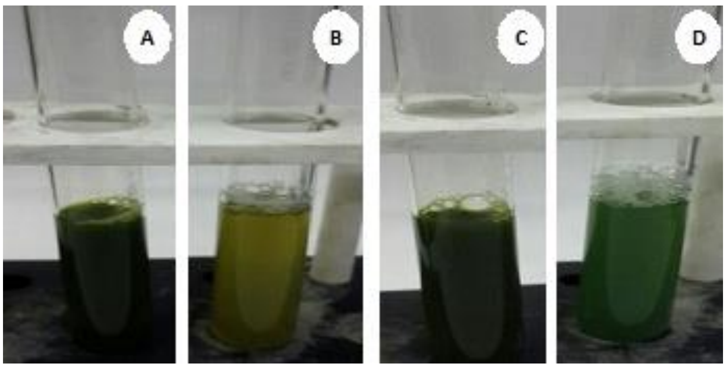

Figure 1: Visual observation after synthesis of nanoparticles a) Plant extract b) Reaction mixture of silver nanoparticles showed colour change c) Plant extract d) Reaction mixture of copper nanoparticles showed colour change

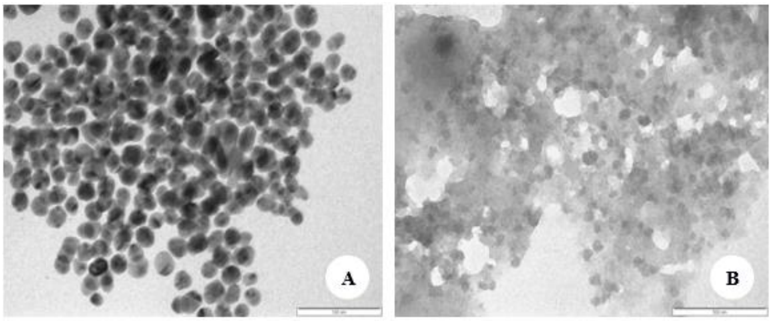

Figure 2: TEM analysis of generated nanoparticles a) Silver nanoparticles and b) Copper nanoparticles

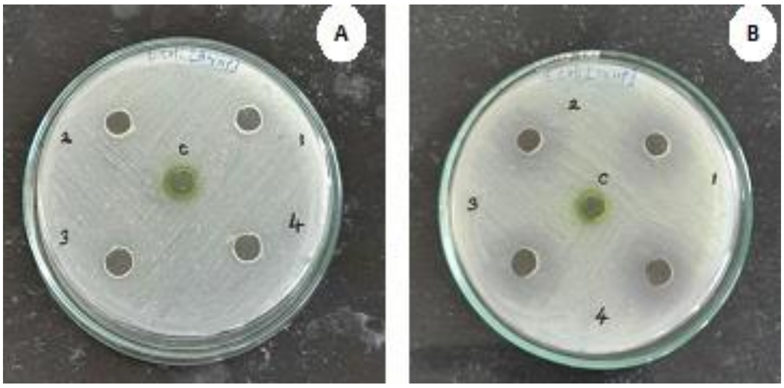

Figure 3: Antibacterial activity against *E. coli* A) Antibacterial activity of silver nanoparticles B) Antibacterial activity of copper nanoparticles. C- Control; Concentrations of nanoparticles: 1- 25 µl, 2- 50 µl, 3- 75 µl, 4- 100 µl

DISCUSSION

Numerous approaches, such as chemical and biological, have been exploited to develop improved methods for the synthesis of nanoparticles. Recently, synthesis of silver nanoparticles using plant extracts has gained popularity[13]. Ashokkumar et al. have reported the synthesis of silver nanoparticles using plant extract of *Abutilon indicum*[14] and Daniel et al. biosynthesized copper and silver nanoparticles using the leaf extract of *Dodonaea viscose*[15].

Similarly, in the present investigation, silver and copper nanoparticles were synthesized using leaf extract of *Cestrum nocturnum* (Figure 1.A and Figure 1.D). A simple and ecofriendly method for the synthesis of silver and copper nanoparticles from a single plant source, *Cestrum nocturnum*, was successfully developed. The generated nanoparticles were characterized using Transmission Electron Microscopy (TEM), which revealed spherical nanoparticles. Copper nanoparticles demonstrated antibacterial efficacy against *E. coli*, and hence, copper nanoparticles might be applicable as a potential antibacterial agent.

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