Biosynthesis, Characterization and Evaluation of Synergistic Antimicrobial Activity by Silver and Copper Nanoparticles Synthesized Using Plant Extract of *Calotropis gigantea*.

[1]Ms. Shridevi Devadiga, [2]Mohammed Mubeen Shaikh, [3]Vikas Gupta, *[4]Dr. Ganesh Lad

[1, 2, 3, 4] Department of Biotechnology, VIVA College (University of Mumbai), Virar (West), Palghar 401303 (MS), India.

Abstract :

Green nanotechnology is a multidisciplinary field that has emerged as a rapidly developing research area, serving as an important, reliable and eco-accommodating technique that emphasizes on making the procedure for synthesizing nanoparticles which are hassle-free, non-hazardous, and environment friendly, in contrast to the currently employed chemical and physical methods. This study reports the synthesis of silver and copper nanoparticles using leaf, flower, and seed extract of *Calotropis gigantea* as reducing, capping and stabilizing agent. A series of reactions was conducted using different types and concentration of plant extracts as well as varying temperature, pH and concentration of metal ions to optimize the reaction condition thus contributing to higher yield of the metal nanoparticles. Visual observation, UV-Visible Spectrophotometry and SEM were used to characterize the synthesized silver and copper nanoparticles. A strong surface plasmon resonance band was observed between 300-400 nm for silver and 300-350nm for copper. Images from the high-resolution Scanning Electron Microscopy (SEM) demonstrated spherical silver nanoparticles with an average size ranging from 65-70nm and copper nanoparticles with a size greater than 100 nm. The antimicrobial action, individual as well as synergistic, of the synthesized silver and copper nanoparticles was tested against representative Gram-positive and Gram-negative bacteria. While individual nanoparticles exhibit antimicrobial effect, no synergistic effect was observed.

***Keywords : Calotropis gigantea*, SEM, variables, copper nanoparticles, silver nanoparticles, antimicrobial activity**

I. INTRODUCTION

The convergence of nanometre sizescale technologies and biological technologies has led to creation of the new field of nanobiotechnology focusing on the creation, manipulation, and use of materials at the nanometre scale for advanced biotechnology (Shah, et. al. 2015). The ability to fabricate and control the structure of nanoparticles allows scientists and engineers to influence the resulting properties design materials to achieve desired properties (Tsuzuki, 2009). Properties such as increased specific surface area is relevant for catalytic activity and for antimicrobial activity such as in silver (Willems, 2005). Nanomaterials also find wide range of application in industries (Roco, 2003; Morrison, 2008). Their unique physicochemical and optoelectronic properties are of particular interest in a number of applications ranging from catalysts, chemical sensors, electronic components, medical diagnostic imaging, and pharmaceutical products (Shah, et. al. 2015).

Production of nanoparticles can be achieved through different methods however biological methods for nanoparticles synthesis using microorganism, enzymes and plant extracts are suggested as possible eco-friendly alternatives (Lee, et. al. 2011). Biological synthesis combines biological principles (i.e., reduction/oxidation) by microbial enzymes or plant phytochemicals with physical and chemical approaches to produce nanosized particles (Shobha, et. al. 2014).

The synthesis of metal nanoparticles using inactivated plant tissue, plant extracts, exudates, and other parts of living plants is a modern alternative for their production. It is a very cost-effective method and therefore a prospective commercial alternative for large-scale production. The variation in composition and concentration of active biomolecules between different plants and their subsequent interaction with aqueous metal ions is believed to be the main contributing factors to the diversity of nanoparticle sizes and shapes produced (Shah, et. al. 2015). Natural hydrocolloids isolated from trees are a new class of potentially economical and environmentally compassionate biomaterial that exhibit a high specificity for the production of nanomaterials (Thekkae and Černík, 2013).

With an increasing interest in the minimization or total elimination of waste and the execution of sustainable processes through the implementation of the fundamental principles of green chemistry, the development of natural and biomimetic approaches for the preparation of nanomaterials is a desirable aspect (Raveendran, et. al. 2003).

Oligodynamic metals, such as silver and copper, have long been utilized as disinfectants for non-spore-forming bacteria and viruses (Thurman, 1988). Copper – an oligodynamic metal, can be combined with silver – another oligodynamic metal, resulting in a synergistic lethal effect on bacterial cells. The positively charged copper ion distorts the cell wall by bonding to negatively charged groups and allowing the silver ion to enter into the cell (Collart, et. al. 2006)

Studies have shown that during the synthesis process, size, shape, stability, and physicochemical properties of the nanoparticles are strongly influenced by a variety of factors. These factors include process parameters (temperature, concentrations, etc.), process kinetics involving the interplay between the metal ion precursors and the reducing agent, and adsorption kinetics involving the stabilizing agent and the nanoparticles (Alexandridis, 2011; Wang et al., 2005).

In this study we report biological synthesis of silver and coper nanoparticles using plant extract. We investigated the effects of reaction conditions such as temperature, concentration of silver nitrate ($AgNO_3$) and copper sulphate ($CuSO_4$) and pH of the reaction mixture on synthesis of copper and silver nanoparticle. The antibacterial activity of synthesized nanoparticles was tested individually as well as synergistically.

II. MATERIALS AND METHODS

2.1 Material

Whole *Calotropis gigantea* plant were collected from waste dumping area in Virar, Palghar district, Maharashtra. The bacterial cultures of *E. coli* and *S. aureus* were procured from Department of Biotechnology, VIVA College, Virar.

2.2 Preparation of plant extract

Fresh whole plants were cleaned using running tap water, followed by distilled water. Aqueous extract of *C. gigantea* was prepared using fresh whole plants. 6 grams of the plant tissue was crushed in 20 ml of Milli-Q water. This extract was filtered through Whatman filter paper (No.1) and the filtrate obtained was diluted to a final volume of 100ml and used to carry out further experiments.

2.3 Synthesis of silver and copper nanoparticles

3 ml of the aqueous extract of leaves, flower, and seed of *C. gigantea* was mixed separtely with 5 ml of 1 mM, 5 mM and 10 mM of $CuSO_4$ and $AgNO_3$ solutions respectively in separate tubes. This setup was incubated in dark to minimize the photoreduction of $AgNO_3$. The effect of variation of different variables on synthesis of nanoparticle of both elements was investigated. The parameters included incubation period (24h, 48h and 72h), pH (5, 7 and 9), temperature (4ºC, 27ºC and 37ºC) and the above mentioned concentrations of $AgNO_3$ and $CuSO_4$.

2.4 Characterization of silver and copper nanoparticles

The presence of synthesized silver and copper nanoparticles were confirmed by sampling the reaction mixtures and obtaining absorption maxima by UV-Vis Spectra, at wavelength of 200-700 nm in Jasco Spectrophotometer V-630. An aliquot of the reaction mixture containing synthesized nanoparticles was used for SEM analysis by FEI Quanta 200 SEM (Thermo Scientific™).

2.5 Antibacterial activity of synthesized silver and copper nanoparticles and evaluation of synergistic action

100 µl of 24 hr old cultures of test organisms of standard density were spread plated on sterile Mueller Hinton agar plates. Sterile Whatman filter paper strips soaked in reaction mixtures containing synthesized nanoparticles were placed on the plates. For synergistic action, two filter paper strips (one soaked in silver nanoparticles and another in copper nanoparticles) were placed perpendicular on top of each other resembling a cross.

III. RESULTS

3.1 Synthesis of nanoparticles (Colour Plates : Figures A and B)

Reduction of the metal ions to metal nanoparticles during exposure to various plant extracts was followed by colour change. Varied intensities of reaction mixtures corresponding to the production of nanoparticles were observed, colour intensifies as the concentration of salt solution and incubation temperature increases. Leaf and seed extracts showed promising results with very low effect of pH on colour change.

3.2 Characterization by UV-Vis spectrophotometer and SEM analysis (Colour Plates : Figures C and D)

The UV-Vis absorption spectrum recorded for the solutions of synthesized silver nanoparticles using leaf (Fig. C1) and seed (Fig. C2) extract showed maximum absorption between 300-400 nm whereas that of copper nanoparticles synthesized using leaf (Fig. C3) and seed (Fig. C4) extract falls between 300-350 nm. Absence of sharp peaks may be due to the polydispersed nature of particles. SEM analysis of the nanoparticles synthesized using leaf extract and 10 mM of salt solution clearly reveals the formation of uniformly small spherical Silver nanoparticles with an average size of 66 nm (Fig. D) whereas Copper nanoparticles are larger structures with size more than 100 nm (hence not shown).

3.3 Antimicrobial analysis and evaluation of synergistic action

Antimicrobial assay of biosynthesized silver nanoparticles against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) microorganisms revealed a fairly good antimicrobial activity of silver nanoparticles against both of the test bacteria. Biosynthesized silver nanoparticles were observed to exhibit better antimicrobial activity on Gram-negative *E. coli* than the Gram-positive *S. aureus*. However, it was observed that copper nanoparticles derived from leaf and seed extract were incapable of inhibiting growth of both the cultures. The synergistic action of both particles was assessed since copper did not inhibit the growth of organisms. However, no synergistic activity was observed between the silver and copper nanoparticles (Fig. E1-E4).

IV. CONCLUSION

The rapid synthesis of stable silver and copper nanoparticles using *C. gigantea* extract solution was demonstrated. Structural and morphological properties of synthesized nanoparticles were characterized. The prepared silver and copper nanoparticles exhibit spherical morphology and particle size in the range of 66 nm and 100 nm respectively by SEM. Synthesized silver nanoparticles also show potential as antimicrobial agents. The present work proves that the *C. gigantea* extracted solution synthesis is a new useful method using cheap precursors for the preparation of silver and copper nanoparticles. This simple, cost effective, time saving and environment friendly synthetic method gives a potential avenue for various applications. The eco-friendly green chemistry approach by the use of these leaf extracts for the synthesis of nanoparticles will increase their economic viability and sustainable management. So, the exploration of the plant systems as the potential nanoparticle production systems has heightened interest in the biological synthesis of nanoparticles.

V. AUTHOR CONTRIBUTIONS

Shridevi Devadiga carried out the laboratory work, Mohammed Mubeen Shaikh co-supervised the laboratory work, Vikas Gupta contributed to the writing, Ganesh Lad conceived and coordinated the study and wrote the manuscript.

All authors gave final approval for publication.

VI. COMPETING INTERESTS

The authors do not have any competing or conflict of interests.

VII. REFERENCES

1] Alexandridis. 2011. "Gold nanoparticle synthesis, morphology control, and stabilization by functional polymers." *Chem. Eng. Technol, 14* 15-38.

2] Collart D, Kepner B, Mehrabi S, Robinson L and Mintz E. 2006. "Efficacy of oligodynamic metals in the control of bacteria growth in humidifier water tanks and mist droplets." *journal of water and health* 1-9.

3] Lee H, Lee G, Jang N, Yun J, Song J and Kim B. 2011. "Biological synthesis of copper nanoparticles using plant extracts." *NSTI-Nanotech Vol 1* 371-374.

4] Morrison T, and Joseph M. 2008. "Nanotechnology in Agriculture and Food." *A Nanoforum report, Institute of Nanotechnology.*

5] Raveendran P, Fu J, Wallen S. 2003. "Completely "green" synthesis and stabilization of metal nanoparticles." *J Am Chem Soc;125(46)* 13940–13941.

6] Roco M. 2003. " Broader societal issue of nanotechnology." *Journal of Nanoparticle Research* 181-189.

7] Shah M, Fawcett D, Sharma S, Tripathy S and Poinern G. 2015. "Green Synthesis of Metallic Nanoparticles via Biological Entities." *Materials 8* 7278-7308.

8] Shobha G, Moses V and Ananda S. 2014. "Biological Synthesis of Copper Nanoparticles and its impact - a Review." *International Journal of Pharmaceutical Science Invention, Volume 3 Issue 8* 28-38.

9] Thekkae V and Černík M. 2013. "Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application." *International Journal of Nanomedicine 2013:8* 889-898.

10] Thurman, R. and Gerba C. 1988. " The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses." *Crit. Rev. Environ. Cont. 18* 295-315.

11] Tsuzuki, T. 2009. "Commercial scale production of inorganic nanoparticles." *International Journal of Nanotechnology,Vol 6* 567-578.

12] Wang L., Chen X, J. Zhan, Chai Y, Yang C, Xu L, Zhuang W, and Jing B. 2005. "Synthesis of gold nano and microplates in hexagonal liquid crystals." *J. Phys. Chem. B* 3189-3194.

13] Willems, van den Wildenberg. 2005. "Roadmap report on nanoparticles." *W&W Espana sl, Barcelona, Spain.*

Colour Plates :

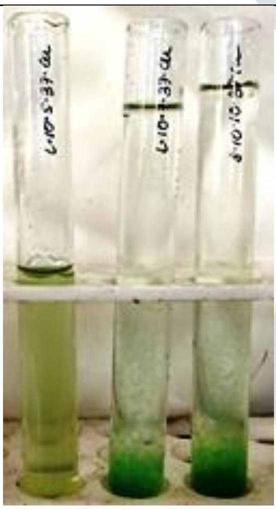
Figure A : Effect of salt concentration on the synthesis of silver nanoparticles from different tissues of *C. gigantea*

A1. From leaf tissue : L→R : 1mM, 5mM, 10mM

A2. From flower tissue : L→R : 10mM, 5mM, 1mM

A3. From seed tissue : L→R : 1mM, 5mM, 10mM

Figure B : Effect of salt concentration on the synthesis of copper nanoparticles from different tissues of *C. gigantea*

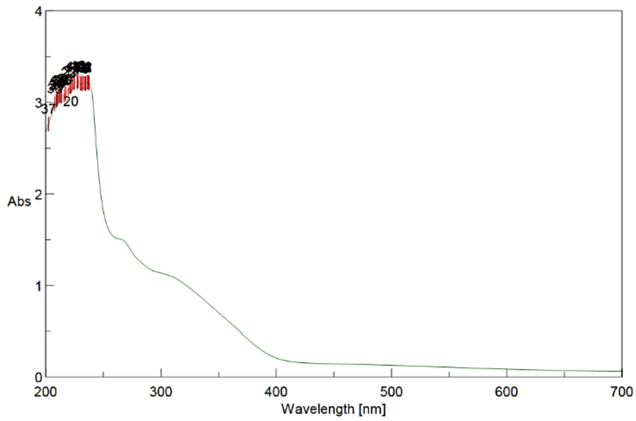


B1. From leaf tissue : L→R : 5mM, 1mM, 10mM

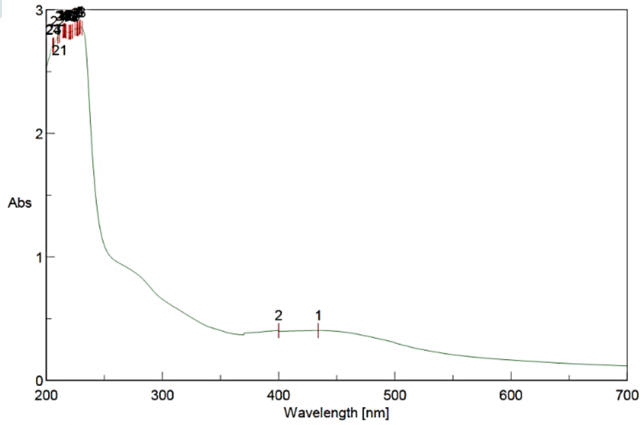
B2. From flower tissue : L→R : 10mM, 5mM, 1mM

B3. From seed tissue : L→R : 1mM, 5mM, 10mM

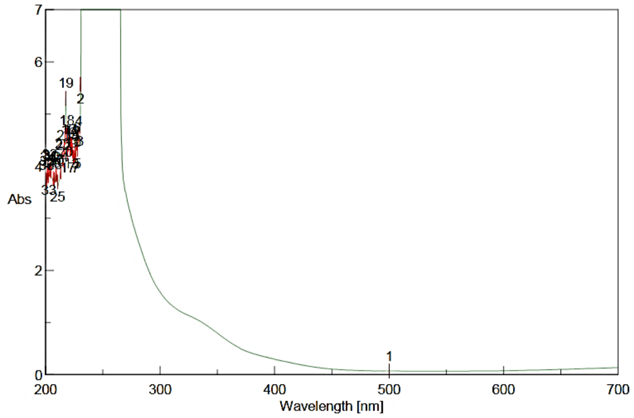
Figure C : UV-Visible Spectrophotometric analysis of silver and copper nanoparticles synthesized using *C. gigantea*



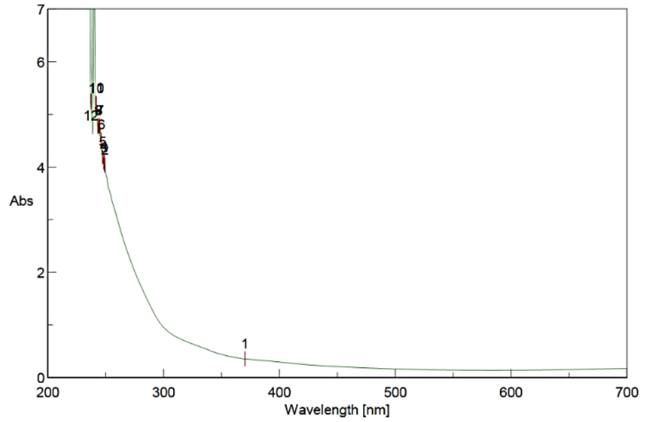
C1. UV-Vis analysis of silver nanoparticles synthesized by aqueous leaf extract of *C. gigantea*



C2. UV-Vis analysis of silver nanoparticles synthesized by aqueous seed extract of *C. gigantea*

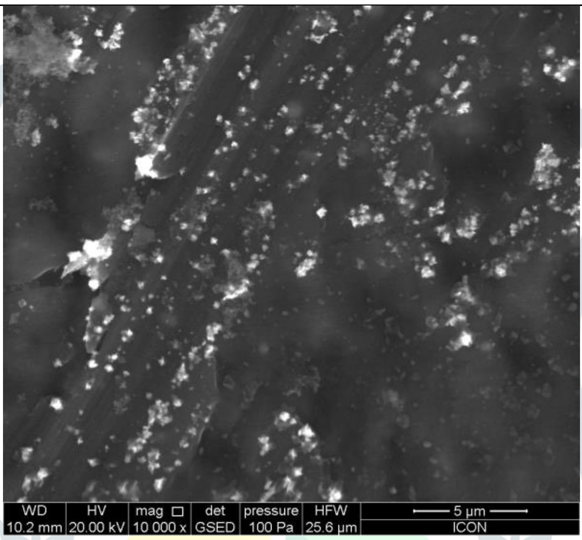


C3. UV-Vis analysis of copper nanoparticles synthesized by aqueous leaf extract of *C. gigantea*



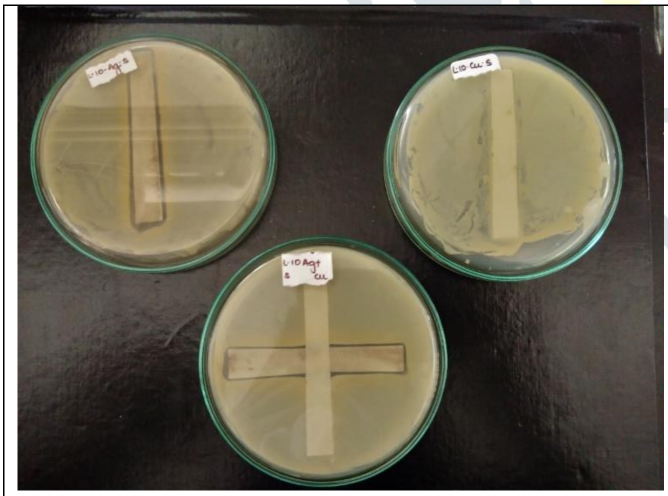
C4. UV-Vis analysis of copper nanoparticles synthesized by aqueous seed extract of *C. gigantea*

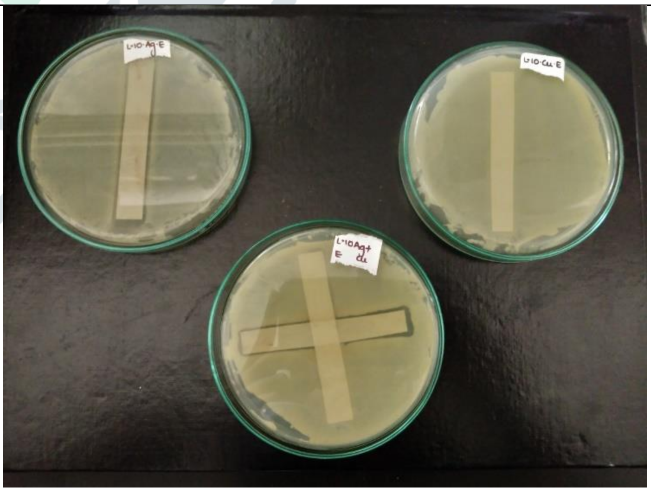
Figure D : SEM analysis of synthesized silver nanoparticles using *C. gigantea*

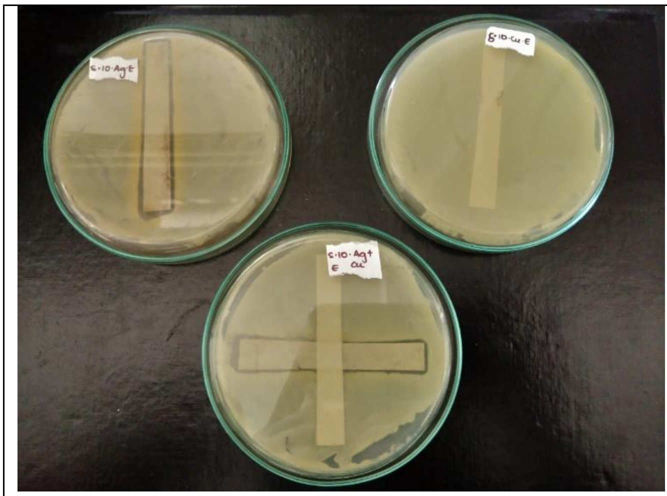


SEM analysis of Silver nanoparticles

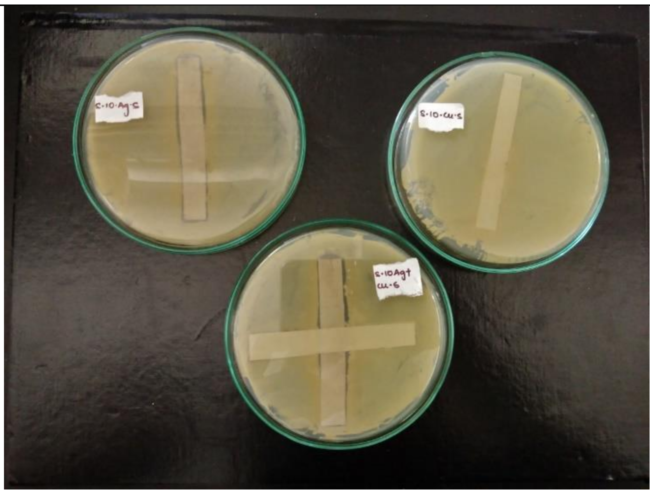
Figure E : Antimicrobial activity of synthesized silver and copper nanoparticles using *C. gigantea*


E1. Antimicrobial activity of Ag and Cu nanoparticles synthesized by leaf extract of *C. gigantea* against *E. coli*


E2. Antimicrobial activity of Ag and Cu nanoparticles synthesized by seed extract of *C. gigantea* against *E. coli*



E3. Antimicrobial activity of Ag and Cu nanoparticles synthesized by leaf extract of *C. gigantea* against *S. aureus*



E4. Antimicrobial activity of Ag and Cu nanoparticles synthesized by seed extract of *C. gigantea* against *S. aureus*