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Enhanced biological activity of Curcumin analogs loaded silver nano particles

Manga Veni P*, K. Devendra Vijaya, G. Durga, P. Bhushanavathi, P. Sarada
Department of Chemistry, St. Josephs College for Women (A), Visakhapatnam
***Corresponding author: E-Mail: mangoveni28@gmail.com**

ABSTRACT

Nano chemistry is an exponentially growing research field in modern science that involves synthesis , characterization and application of nanoparticles with different sizes and shapes. The study on the application of nano materials in various domains such as medicine, industry, consumer products, food technology, environment and agricultural fields is an emerging field of research and development. Among this broad spectrum of applications, biological screening of synthesized nanoparticles is extensively carried and presented in this paper. The present work involves synthesis of curcumin analogs- 1, 7-bis (substituted phenyl) 1, 6-heptadiene 3, 5-dione loaded Silver nanoparticles by chemical reduction. Silver nitrate was incorporated as metal precursor and sodium borohydride as a reducing agent. The formation of silver nanoparticles was monitored by UV-Visible absorption spectroscopy. The curcumin analogs loaded nanoparticles have shown enhanced activity when screened against *Vibrio cholera. Vibrio alginolyticus, Vibrio fluvais, Vibrio parahaemolyticus.*

KEY WORDS: Silver nano particles, enhanced biological activity, *Vibrio Species.*

1. INTRODUCTION

Nanotechnology addresses the synthesis of nano materials at atomic level to attain unique surface property. The confluence of nanotechnology and biology can minimize many biomedical problems, and can revolutionize the field of health and medicine. Silver salts are widely used in wound dressing and other therapeutics. However the therapeutic dosage involved with nanoparticles was minimized to a large extent. The diversified application of Silver in its nano size addressed the number of biological activities. Despite of great progress in development of antibacterial agents, there are still special needs to find new antibacterial agents due to development of multidrug resistant bacteria. Nano silver is an effective killing agent in a broad spectrum of Gram-negative and Gram-positive bacteria including antibiotic-resistant strains. Gram-negative bacteria include genera such as *Acinetobacter*, *Escherichia, Pseudomonas*, *Salmonella*, and *Vibrio*. Antimicrobial activity of silver nanoparticles with Gram-negative bacteria, irrespective of their size shape and concentration. However to develop stable nano particles and to retain their high activity it is necessary to terminate the particle growth and to stabilize the surface. Protection has been achieved through the addition of capping agents by immobilization of solid materials with high specific areas. The capping agents employed for this purpose are mainly polymeric substrates of any long chain alkyl substrates with polar head that can bind to the metal through kelation. Some selective substrates are loaded to stabilize the nanoparticles and to prevent them from aggregation during catalytic transformation. Polymers are considered to be the best stabilizing agents because of they can cover large surface area of nanoparticles. Curcumin has been reported as potent drug for its antibacterial, antiviral, antifungal, and antimalarial action. Due to extended antimicrobial activity of curcumin and non toxic nature even at high doses (12g/day) assessed by clinical trials in human, it was used as a structural sample to design the new antimicrobial agents with modified and increased antimicrobial activities through the synthesis of various derivatives related to curcumin. However curcumin has very poor bioavailability and insufficient solubility in aqueous solvents leading to poor absorption, fast metabolism, and quick systemic elimination. To overcome this obstacle, nanocarriers like curcumin-loaded PLGA (poly lactideco-glycolide) and curcumin nanoparticles formulation were investigated and their better bioactivity and bioavailability as well as increased cellular uptake compared to curcumin were reported. 1,7-bis(2,5-dimethoxy phenyl)1,6-heptadiene 3,5-dione loaded Silver nanoparticles were synthesized to demonstrate the enhanced activity as the combinational drug delivery carrier in the present paper.

Materials and Instruments: All the reagents used were of AR grade. $AgNO_3$ from ALDRICH, $NaBH_4$ was obtained from Merck, India. New Curcumin analogues with >90% HPLC purity, Organic-free 3D water were used for experimentation. The UV Visible spectrometric determination was carried out on Perkin ElmerLamda750. The spectrum was recorded for each sample, and the solutions were taken into 1 cm well stoppered quartz cuvette. Fourier transform infrared (FTIR) spectral characteristic of the samples were collected on Shimadzu FTIR spectrometer using KBr pellets.

Synthesis of (1E, 6E)-1, 7-bis (2, 3, 4-dimethoxy phenyl) hepta-1,6-diene-3,5-dione capped silver nanoparticles: (AgNP): About 2.5 ml of 10^{-2} M $AgNO_3$ solution was added to 75 ml of triple distilled organic free water. 5 ml of 10^{-2} M (1E, 6E)-1,7-bis(2,3,4-trimethoxyphenyl)hepta-1,6-diene-3,5-dione compound synthesized initially was dissolved in methanol and added as stabilizer to the above solution with the constant stirring. After 10 min. of mixing, 2.5 ml of 10^{-2} M Potassium Iodide (KI) was added slowly drop by drop. Pale yellowish green silver iodide (Ag I) colloidal suspension was appeared. Under vigorous stirring, a total of about 2 mg $NaBH_4$ (0.01 M) was added drop by drop to AgI colloidal solution,

and the reaction mixture imparted rapid color charges to yellowish white green, green to nut brown, brown to grey and then finally to black. At every instant color change, UV absorption was periodically measured. The final black color confirms the completion of reduction and formation of nanoparticles. The formed silver nanoparticles were treated with 1ml of 0.001 M sodium citrate solution for further stabilization and carefully collected by centrifugation. Black colored silver nanoparticles were well dried and subjected for experimental characterization.

By UV-visible spectroscopy: UV visible spectroscopy is one of the most suitable techniques for the characterization of Silver nanoparticle (AgNP). AgNP shows strong absorption band due to large surface area when compared with the bulk metal and surface plasma resonances makes AgNP to shift to longer wavelengths with increasing particle size. The gradual increase in the wavelength was accomplished by the colloidal suspension changes from pale yellow to green, green to nut brown. The position and shape of the Plasmon absorption band of metal nano clusters are strongly dependent on the particle size, dielectric medium and surface-adsorbed species. The UV- Visible spectrum of AgNP was recorded at 422nm.

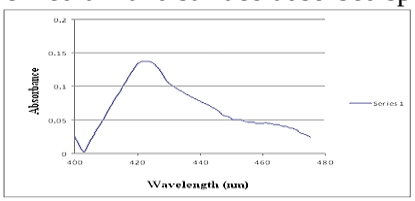

Figure.1. UV visible spectrum of AgNP

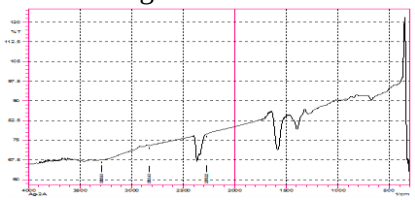

Figure.2. IR spectrum of AgNP

(b) By FT –IR studies: IR spectrum of **AgNP** showed two prominent bands at 1650 cm^{-1}, 2178 cm^{-1}, 2833 cm^{-1} representing the carbonyl group and aromatic C-H stretching vibrations. These stretching vibrations are invariant with respect to the formation of the adsorption at the nanoparticles surface.

(C) Biological activity: Vibriosis is a bacterial disease and is responsible for mortality of cultured shrimp worldwide. It has been reported that penaeid shrimp culture systems severely affected by *Vibrio Cholera Vibrio harveyi, V. splendidus, V.parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus, V. campbelli, V. fischeri, V. damsella, V. pelagicus, V. orientalis,V. fluvialis, V. ordalii, V. mediterrani, V. logei* etc. Out of the above, *VibrioCholera Vibrio harveyi, V. alginolyticus, V. splendidus* species are adopted for screening. Luminous Vibrio was gathered from hatchery rearing water. In these processes Agar diffusion method cup plate, cylinder and paper disc methods can be employed. By cup plate method Curcumin analog (2,3,4 TMHDD) and capped nanoparticles were screened over TCBSA (Thio-sulphate Citrate Bile Salts sucrose agar medium). The sub cultures of *Vibrio Cholera, Vibrio harveyi, Vibrio alginolyticus, Vibrio splendidus* were grown over night at 37^0C. The (1E, 6E)-1,7-bis (2,3,4-trimethoxyphenyl) hepta-1,6-diene-3,5-dione (2,3,4 TMHDD)loaded silver nanoparticles and pure (1E, 6E)-1,7-bis (2,3,4-trimethoxyphenyl) hepta-1,6-diene-3,5-dione were screened for its activity at two concentrations (0.50, 1.0 µg/ml) and was compared with the standard drug tetracycline. The inhibition zones were presented in Table.1.

Table.1. The inhibition zones 2, 3, 4 TMHDD and AgNP

Pathogens	Causing disease	2,3,4TMHD at 1µg/ml	AgNP at 1 µg/ml
Vibrio Cholera	Cholera	16	9
Vibrio harveyi	White gut disease	14	8
V. splendidus	WGD, Loose shell syndrome(LSS)	20	8
V. alginolyticus	LSS, WGD, Red disease	18	6

2. CONCLUSION

1,7-bis(2,3,4-trinitro phenyl)1,6-heptadiene 3,5-dione loaded silver nanoparticles have potent inhibition at 1 µg/ml clearly demonstates the combinational drug therapy has great demand. The enhanced activity of silver nanoparticles even at 1 µg/m can cause complete distruction at the cellular level.The non toxic combinational therapy has shown significanct importance in inhibition.

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