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Characterization and evaluation of anti-biofilm effect of green synthesized copper nanoparticles

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Abstract

Copper nanoparticles (CuNPs) have been synthesized using two stage chemical reduction method, where a separate reducing agent Hydrazine Hydrate (HH) and a separate stabilizing agent Gum Kondagogu extract are used. The synthesized CuNPs are characterized by using Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), UV visible spectroscopy, X-Ray Diffraction (XRD), FT-Raman spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR) experimental methods. These experimental results reveal the formation of stable FCC crystalline CuNPs with thin Gum Kondagogu extract layer around their surface. Biofilm is the major target for the development of drugs and it is related to most preferred type of highly infectious factor of pathogenic microorganisms. A biofilm promotes persistence of bacteria by resisting antibiotic treatment. In the present study, anti-biofilm effect of Gum Kondagogu extract stabilized CuNPs against clinical isolate Klebsiella Pneumoniae (Gram -ve, ATCC 27736) bacteria is tested. SEM results reveal effective anti-biofilm effect on the CuNPs film. The present study would encourage the development of anti bacterial coated medical devices against pathogenic microorganisms.

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Keywords: CuNPs; Gum Kondagogu extract; Klebsiella Pneumoniae; Anti-biofilm effect

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1. Introduction

Green synthesis of metal nanoparticles has attracted many researchers as it is simple eco-friendly approach that aims to reduce the usage of substances hazardous to human health and environment (1). Green synthesis studies have mainly focused on the nanoparticles like silver, gold etc. due to their novel properties such as intense plasmon resonance, chemical and bio-stability, catalytic activity, antibacterial activity etc. However, the cost of synthesis of these nanoparticles is very high (2). Copper nanoparticles (CuNPs) are considered to be alternative to these nanoparticles as it is cheaper besides exhibiting novel properties like high conductivity, catalytic activity and antibacterial activity (3). Though CuNPs can be synthesized via different experimental techniques chemical reduction method is preferred over all other as it is simple and economical. But, the green synthesis of CuNPs directly from various parts of the plants is not easy via single stage chemical reduction method as it is prone to the formation of copper oxide. However, the CuNPs can be synthesized using two stage chemical reduction method where a separate reducing agent and a separate stabilizing agent are used. In two stage chemical reduction method plant extracts are used as stabilizing agents and this method is preferred for the synthesis of CuNPs as it efficiently avoids the formation of copper oxide (4). In the present study Gum Kondagogu is used as stabilizer during the synthesis of CuNPs. Gum Kondagogu is a natural polysaccharide found in the exudates from the tree Cochlospermum Gossypium which is rich in protein and soluble fibre (5).

Biofilm is involved in the development of clinical infection and exhibits resistance to antibacterial agents. When biofilms form, antibiotics become ineffective due to their relative impermeability, various physiological statuses of microorganisms, super populations of persistent strains etc. Metal NPs has the potential to prevent the formation of these lives threatening biofilms on life supporting devices (6). CuNPs have been the focus of increasing interest due to their broad spectrum of antibacterial activity. In the present study, anti-biofilm effect of Gum Kondagogu extract stabilized CuNPs against clinical isolate of Klebsiella pneumoniae (Gram -, ATCC 27736) has been studied.

2. Experimental

Gum Kondagogu samples were collected from Girijan Co-operative Corporation, Government of Telangana Undertaking, Hyderabad, India. Gum Kondagogu was powdered to obtain a fine and uniform sample. Gum Kondagogu powder (4 g) was accurately weighed, and dispensed into a clean glass beaker containing 1 L of Millipore water. The whole gum solution was kept on a magnetic stirrer at room temperature and gently stirred overnight. Later, the gum solution was allowed to stand at room temperature at 30°C for 12 hrs to separate any un-dissolved matter. The gum solution was filtered through a sintered glass funnel. The clear solution was freeze-dried and stored until further use for preparation of CuNPs. The starting chemicals used for the preparation of CuNPs are copper sulphate, L-Ascorbic acid, NaOH and Hydrazine Hydrate (HH). All chemicals used were of analytical reagent grade. The solutions were made with Millipore water.

In the present study, CuNPs were synthesized by two stage chemical reduction method. Copper salt was used as basic precursor, Gum Kondagogu extract as a stabilizer, HH as a reducing agent and L-Ascorbic acid as an anti-oxidant agent. NaOH was used as a catalyst and also to adjust the pH to 12. Copper sulphate, L-Ascorbic acid and Gum Kondagogu extract solutions were prepared separately using Millipore water. The solutions of Gum Kondagogu extract and L-Ascorbic acid were added to copper sulphate solution while stirring. Then the solutions of HH and NaOH were added to the mixed copper salt solution under stirring. Then the initial blue colour of the reaction mixture eventually turns in to brown-black colour. Stirring was continued for another 1 hr. to complete the reaction. The precipitate was washed twice with methanol after filtration and then dried to obtain CuNPs.

Morphology and size of the CuNPs were investigated using Transmission Electron Microscope (EM-Phillips equipment) and Scanning Electron Microscope (SEM-Hitachi S-3400N equipment). The UV-Visible spectrometer (Lab India Instruments Pvt. Ltd, Lab India UV- 3000+) was used to study the surface plasmon peak of colloidal dispersion of CuNPs. All spectra were corrected against the background spectrum of water as reference. XRD patterns of CuNPs were recorded using Philips X-ray diffractometer coupled with graphite monochrometer. The Crystallite size of the CuNPs was calculated using Williamson – Hall equation given as following.