Biosynthesis of silver nanoparticles by natural precursor from clove and their antimicrobial activity*

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Abstract: Silver nanoparticles (AgNPs) have attracted the attention of researchers because of their unique properties and applications in various fields, such as medicine, catalysis, textile engineering, and pollution treatment. The green synthesis of AgNPs has many advantages, such as less time requirement, highly stable AgNPs, better control over crystal growth, morphology, ease for scale up, and economic viability. Syzygium aromaticum (clove) was used for the extracellular biosynthesis of AgNPs. Eugenols are the active biomolecules present in clove, responsible for the bioreduction of AgNO$_3$ (Ag$^+$) leading to the formation and capping of AgNPs (Ag$^0$). One molecule of eugenol releases two electrons and these two electrons will be taken by 2 Ag$^+$ ions and these will get reduced to 2 Ag$^0$. The synthesis of AgNPs was confirmed by the appearance of brown colour. The synthesized AgNPs were characterised by various techniques, such as UV-VIS spectroscopy, transmission electron microscopy, X-ray diffraction and Fourier transformed infrared spectroscopy. The synthesised AgNPs have $\lambda_{\text{max}}$ of 440 nm. It was evaluated that the AgNPs were biphasic in nature (cubic + hexagonal) with an average size of 50.0 nm. The synthesized AgNPs showed significant antimicrobial activity against Bacillus cereus NCDC 240 as they are nano-sized and have high surface area to volume ratio. AgNPs inhibit the growth of bacteria by various ways, such as by disrupting the cell membrane of bacteria, uncoupling the oxidative phosphorylation, inhibiting the DNA replication, forming free radicals and affecting the cellular signalling of bacteria leading to cell death.

Key words: Syzygium aromaticum; nanoparticles; greensynthesis; Bacillus cereus.

Abbreviations: AgNPs, silver nanoparticles; DLS, dynamic light scattering; FTIR, Fourier transformed infrared; TEM, transmission electron microscopy; XRD, X-ray diffraction.

Introduction

Nanotechnology is concerned with the synthesis of nanoparticles of variable sizes, shapes and chemical composition and their use for human benefits. Nanoparticles are made up of a variety of materials; most commonly used are metal oxides, such as titanium, zinc and iron oxides, other widely used forms being gold and silver metal nanoparticles. Silver nanoparticles (AgNPs) have emerged as an outstanding product from the field of nanotechnology because of their unique properties such as good conductivity, chemical stability, catalytic properties, electrical and magnetic properties, which can be incorporated into antimicrobial applications, biosensor materials, composite fibres, cosmetic products and electronic components (Mukherjee et al. 2001; Sondi & Salopek-Sondi 2004). These can be prepared by chemical methods, such as chemical reduction, electrochemical techniques and photo-chemical reduction, which suffer from various limitations such as low yield, cost ineffectiveness, toxicity and instability (Huang et al. 2007; Sharma et al. 2009). Therefore, there is an increasing need to develop high yielding, cost effective, eco-friendly and nontoxic procedures for synthesis of AgNPs. In this view, biosynthesis or green synthesis have attracted a great deal of interest. Green synthesis of AgNPs offers all the above mentioned advantages and can be done by using a variety of microorganisms, such as Klebsiella pneumoniae, Bacillus subtilis, Fusarium semitectum, Penicillium fellutanum and Candida sp. (Basavaraja et al. 2008; Kathiresan et al. 2009; Mokhtari et al. 2009; Panacek et al. 2009; Saiffudin et al. 2009). But these sources possess certain limitations, e.g., strict growth requirements, long growth periods and difficult downstream processing.

Plant and plant extracts have shown a great potential for the synthesis of AgNPs because they possess...
a broad variety of metabolites like flavones, ketones, terpenoids, aldehydes, amides and carboxylic acids responsible for the bioreduction process involved in the synthesis. Moreover, advantages associated with them, like ease of availability, non-pathogenicity, rapid synthesis and easy recovery, make them a preferred choice over the microbial sources. Several plant sources used so far include *Cinnamomum camphora*, *Aloe vera*, *Cycas* leaf extract, *Jatropha curcas*, *Magnifera indica* and *Syzygium aromaticum* (Chandra et al. 2006; Huang et al. 2007; Bar et al. 2009; Philip 2009; Kumar et al. 2010). Among them the use of *S. aromaticum*, which is commonly known as clove, is justified by easy availability of dried clove buds throughout the year and strong reducing capability of its dominant compound eugenol (Singh et al. 2010).

AgNPs have strong antimicrobial activities and can attack at the same time a broad range of targets in microorganisms, such as proteins with thiol groups, cell walls and cell membranes. AgNPs show antimicrobial activities against various infectious organisms, e.g., *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio cholerae* (Kim et al. 2009; Narasimha et al. 2011). Due to their antimicrobial activity, AgNPs can play a promising role in food packaging system to prevent the growth of fouling contaminants and to improve the shelf life of food. In therapeutic treatment AgNPs display cytoprotective activity against HIV-1 infected cells. In water and air filters they can be incorporated into apparels, footwears, paints, wound dressings, appliances, cosmetics and plastics (Sun et al. 2005; Sharma et al. 2009; Kannan & Subbalaxmi 2011).

The present work reports the green synthesis of AgNPs from natural precursor *Syzygium aromaticum* to check their antimicrobial potential using *Bacillus cereus*.

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**Material and methods**

**Material**

*Bacillus cereus* NCDC 240 was procured from National Collection of Dairy Cultures (Karnal, India). *Syzygium aromaticum* was obtained from Savour India Private Ltd. (New Delhi, India). Silver nitrate, nutrient broth and HPLC grade water were obtained from HiMedia and Merck (Mumbai, India) and were of analytical grade.

**Biosynthesis of silver nanoparticles**

For AgNPs synthesis, *S. aromaticum* extract was prepared by agitating a mixture of 2 g *S. aromaticum* powder in 100 mL deionised water at 27 °C for 4 h at 100 rpm. Thereafter, the contents were filtered and the filtrate was heated at 40–45 °C to obtain a dry-brown-coloured residue. This residue was dissolved in 100 mL HPLC-grade water with constant stirring followed by filtration to get a dark-brown-coloured clear extract. For synthesis of AgNPs, 10% (v/v) *S. aromaticum* extract was added to 1 mM AgNO₃ solution and incubated at 27 °C at 100 rpm for 1 h followed by its centrifugation at 8,000 rpm for 10 min for retrieval of nanoparticles.

**Characterization of silver nanoparticles**

The AgNPs were characterised by UV-VIS spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS), X-ray diffraction (XRD) and Fourier transformed infrared (FTIR) spectroscopy. The reduction of metallic Ag⁺ ions was monitored by measuring the UV-VIS spectrum for which small aliquots were withdrawn from the reaction mixture at intervals of 1 min, 5 min, 30 min, 1 h and 18 h and a spectrum was recorded between 300–700 nm on EL 2371 UV-VIS spectrophotometer. The morphology of nanoparticles was analysed with Hitachi 7500 transmission electron microscope at an accelerating voltage of 80 kV and DLS of AgNPs was obtained using Nano ZS Instrument (Malvern, UK). XRD was done on Bruker axs D8 diffractometer at a 40 kV voltage and 40 mA current with Cu Kα radiation in a θ–2θ configuration. The FTIR spectra of AgNPs was recorded on a Perkin Elmer Spectrum 400 FTIR Spectrometer.

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**Fig. 1. Absorption spectra of AgNPs.**