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ORIGINAL ARTICLE

Characterization of biosynthesized gold nanoparticles from aqueous extract of *Chlorella vulgaris* and their anti-pathogenic properties

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Abstract In this study, biosynthesis of self-assembled gold nanoparticles (GNPs) was accomplished using an aqueous extract of green microalga, *Chlorella vulgaris*. The optical, physical, chemical and bactericidal properties of the GNPs were investigated to identify their average shape and size, crystal nature, surface chemistry and toxicity, via UV–visible spectroscopy, scanning electron microscopy, transmission electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy and antimicrobial activity. The sizes of the spherical self-assembled cores of the synthesized GNPs ranged from 2 to 10 nm. The XRD patterns showed a (111) preferential orientation and the crystalline nature of the GNPs. The results of the FTIR analysis suggested that the peptides, proteins, phenol and flavonoid carried out the dual function of effective Au III reduction and successful capping of the GNPs. Human pathogen *Candida albicans* and *Staphylococcus aureus* were susceptible to synthesized aqueous GNPs. Thus, biosynthesis, stabilization and self-assembly of the GNPs by *Chlorella vulgaris* extract can be an example of green chemistry and effective drug in the medicinal field.

Keywords GNPs · Green synthesis · Toxicity · Human pathogens

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Introduction

Gold is an important material for various applications in nanoscale devices and technologies due to its chemical inertness and resistance to surface oxidation. Meanwhile, size-controlled synthesis of metal nanoparticles is critical for its application in various fields such as electronics, optics, optoelectronics and biosensors (Dutta et al. 2004). Though a wide variety of physical and chemical processes had been developed for the synthesis of metal nanoparticles (Dahl et al. 2007; Kumar and Yadav 2009), the methods are expensive and requires the use of toxic and aggressive chemicals as reducing and capping agents (Hutchison 2008). Therefore, green chemistry should be integrated into nanotechnologies, especially when nanoparticles are to be used in medical applications which include imaging, drug delivery, disinfection and tissue repair (Albrecht et al. 2006). The manufacturing of nanoparticles under totally 'green' principles can be achieved via the selection of an environmentally acceptable solvent system with eco-friendly reducing and stabilizing agents (Xie et al. 2007). Biological approaches to nanoparticle synthesis have been suggested as valuable alternatives to physical and chemical methods (Bhattacharya et al. 2005). Most of the studies involve biomolecules (proteins, amino acids and carbohydrates), whole cells of various microorganisms (bacteria, fungi and algae) or plant resources (roots, leaves, flowers, bark powders, seeds, roots and fruits) for the synthesis of metal nanoparticles (Dahl Maddux and Hutchison 2007; Mohanpuria et al. 2008; Huang et al. 2009; Kumar and Yadav 2009). In particular, naturally grown plant species which are a vital source of phytochemicals may serve as environmentally benign reservoirs for the production of metallic nanoparticles (Nune et al. 2009). In addition, they do not require elaborate processes such as intracellular

synthesis and multiple purification steps or the maintenance of microbial cell cultures.

The green microalga, *Chlorella vulgaris* is widely known as a single cell protein and used in the food, medicine and manufacturing industries. It is a rich source of biologically active compounds such as chlorophylls, carotenoids, astaxanthin, phenols, flavonoids, protein, vitamins and minerals (Faulkner 2000). In addition, its phytochemicals include hydroxyl, carboxyl and amino functional groups that serve both as effective metal-reducing agents and as capping agents to provide a robust coating on the metal nanoparticles in a single step. In this study, a simple environmentally friendly and self-sufficient biosynthetic approach was investigated for the preparation of gold nanoparticles (GNPs) with *C. vulgaris* and its bactericidal activity was studied. The use of the green algae phytochemicals serves an easy and environmentally benign method of preparing GNPs. GNPs' synthesis by mixing an aqueous solution of chloroauric acid with cell-free aqueous extract of *C. vulgaris* complies with the green chemistry principles of using safe aqueous phytochemicals-based synthesis and extends its possible usage in the isotropy of spherical nanoparticles. The obtained GNPs were comprehensively characterized for their average core size, morphology, purity, surface capping, crystal structure, and optical and bactericidal properties.

Materials and methods

The freshwater green algal strains of *C. vulgaris* were collected from Algal Culture Collection, Center for Advanced Studies in Botany, University of Madras, Chennai, India and was inoculated in a Bold's Basal medium. The culture was maintained at 24 ± 1 °C in a thermostatically controlled room and illuminated with cool fluorescence lamps at an intensity of 2,000 lux in a 12:12 h light dark regime. In the exponential log phase, when the pigment, protein and carbohydrate were measured to be maximum, the cells were harvested. The collected cells were washed with double-distilled water and sonicated using ultrasonic vibration at 30 % amplitude for 20 min to release the water-soluble biomolecules. The aqueous biomolecules were subjected to centrifugation (three to four times, at 5 °C, 14,000 rpm, for 30 min) to remove any cell debris. The obtained *C. vulgaris* cell-free extract was diluted through a series of dilutions with 1 mM $HAuCl_4$. The reaction mixtures (10 mL) were put aside at 37 °C and the reduction of the gold ions started at 1 mM $HAuCl_4$.

The bioreduction and optical properties of the freshly prepared GNPs were investigated by measuring the UV–Vis spectrum between 400 and 700 nm in a 10 mm path length quartz cuvette with a 1 nm resolution (UV–Vis, Beckman DU 64). TEM samples were prepared via drop casting on carbon-coated lacey films. The films were dried prior to the measurement of the GNPs and operated at an accelerating voltage of 30 kV using a CCD camera (Hitachi H-7600 AMT V600). The freshly prepared GNPs with a glass substrate were subjected to XRD to measure the pattern (Model D/Max-2500) and scanning was executed in a 2θ region from 30 to 80°. The pattern was recorded using Cu–K_α radiation with a wavelength (λ) of 1.5406 Å at a tube voltage of 40 kV and a tube current of 30 mA. FTIR analysis was carried out after the removal of the free biomolecules that were not adsorbed by the nanoparticles after repeated centrifugation and re-dispersion in water. Antimicrobial activity of the synthesized GNPs was carried out by agar well diffusion method in triplicate (Bauer et al. 1986) against five human pathogens: *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

Result and discussion

Synthesis of gold nanoparticles

The development of economic and reliable biosynthetic method has been focused for the production of nanostructured gold particles using *C. vulgaris* and analysis of its antimicrobial activity. Aqueous extract of *C. vulgaris* is a reservoir of phytochemicals including pigments, astaxanthins, organic acids, amino acids, phenol, flavonoids, peptide and protein. In addition, the presence of carbohydrates (polysaccharides, oligosaccharides and reducing sugars) in the extract provides synergistic reducing power for the rapid transformation of chloroaurate ions into GNPs.

Characterization of gold nanoparticles

Different fractions of *C. vulgaris* cell-free extract reacted with $HAuCl_4$ (1 mM) at 37 °C. The analysis of the UV–visible spectrophotometric data confirmed that the surface plasma resonance (SPR) band was located at 530 nm for the GNPs synthesized (Fig. 1a). The conversion of gold ions into GNPs was found to be 90–95 % at 37 °C. The colors of all the test reaction mixtures changed from yellow to red, then black and the reaction completion was found to be dependent on the concentrations of the *C. vulgaris* fractions (Fig. 1b). The dark red color of the reaction mixture in all the studied test fractions indicates the formation of the GNPs. The visible color is the effect of the resonant light interaction with the GNPs via the excitation of the surface plasmon due to the light scattering and absorption as determined by the size of the GNPs (Higuchi

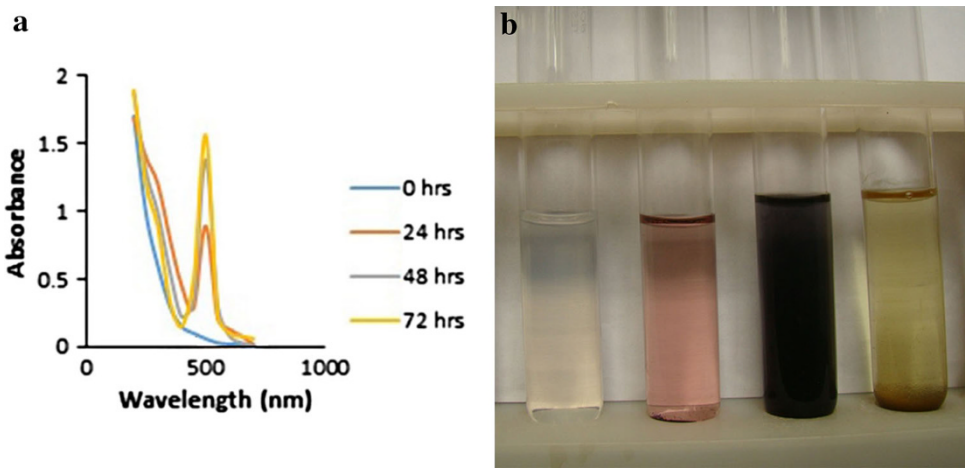

Fig. 1 **a** UV–visible spectra and **b** reaction mixture of gold nanoparticles synthesized through the reduction of aqueous chloroauric acid at different dilutions

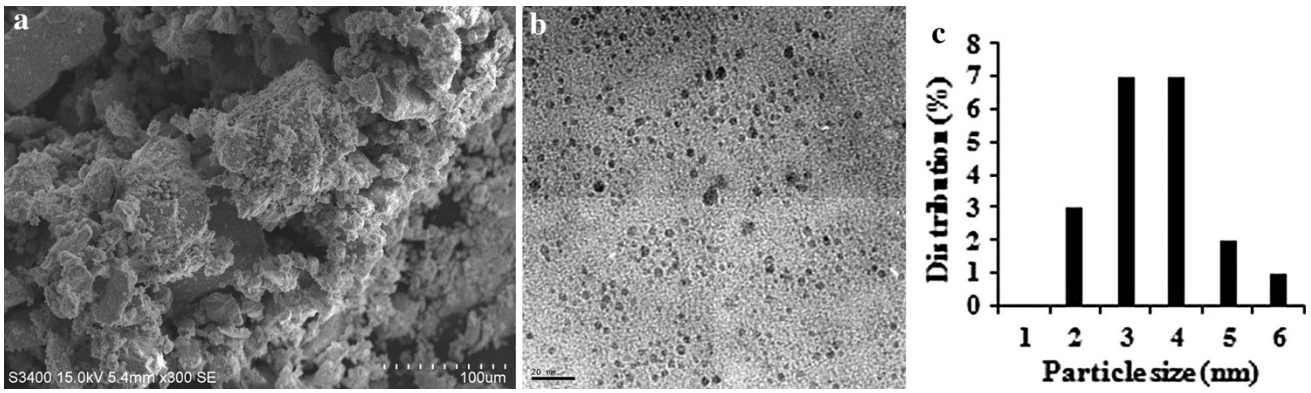

Fig. 2 **a** SEM and **b** TEM micrograph of GNPs and **c** their particle size distribution

et al. 2007; Tripathy et al. 2010). The proposed green synthesis method for GNPs was found to be constructive and extremely reproducible.

The synthesized GNPs were subjected to detailed characterization; to determine their sizes, morphologies, physical properties (e.g., optical properties and crystal structure), and surface chemistry and its toxicity to bacterial cells. The sizes and morphologies of the GNPs were imaged via SEM and TEM, respectively (Fig. 2a, b). The SEM and TEM analysis revealed that the GNPs had an identical nature with an average size of about 10–2 nm and there was a spatial array of self-assembled nanostructures throughout the imaging (Fig. 2c).

The key interactions of the GNPs were predicted to have a lateral driving force and formed self-assembled nanostructures. As previously reported, the aggregation and self-assembled nanostructures could be induced by particular biological interactions, and the peptides present on the NP surfaces could be responsible for connecting the nanoparticles, thus forming fibril nanostructures via the dipole–dipole interaction pattern (Shankar et al. 2004). There is an increasing interest in the development of a self-assembly procedure and in the production of complex nanostructures to obtain new material properties (Daniel and Astruc 2004).

The crystalline nature of the GNPs was evaluated via XRD. The angular positions of the diffracted Bragg peaks were observed. It was confirmed that the nanoparticles have a face-centered cubic structure with a lattice constant of 4.05 Å. The Bragg peaks equivalent to (111), (200), (220) and (311) demonstrate the formation of crystalline GNPs (Fig. 3a). The bimolecular crystallization during the formation of the metal nanoparticles has been reported (Shankar et al. 2004). FTIR studies were carried out to identify the surface properties of the GNPs. A major peak at 1,635 cm^{-1} (Fig. 3b) corresponds to amide-I and amide-II bonding from the capped peptides; similar results have been reported (Xie et al. 2007). The stabilization of the inorganic surface could have taken place due to the steric and/or electrostatic barriers on the nanoparticles' surfaces (Mulvaney 1996).

Based on the GNPs' surface chemistry, pigments, polysaccharides and the peptides and/or proteins of *C. vulgaris* extract was found to be the key biomolecules

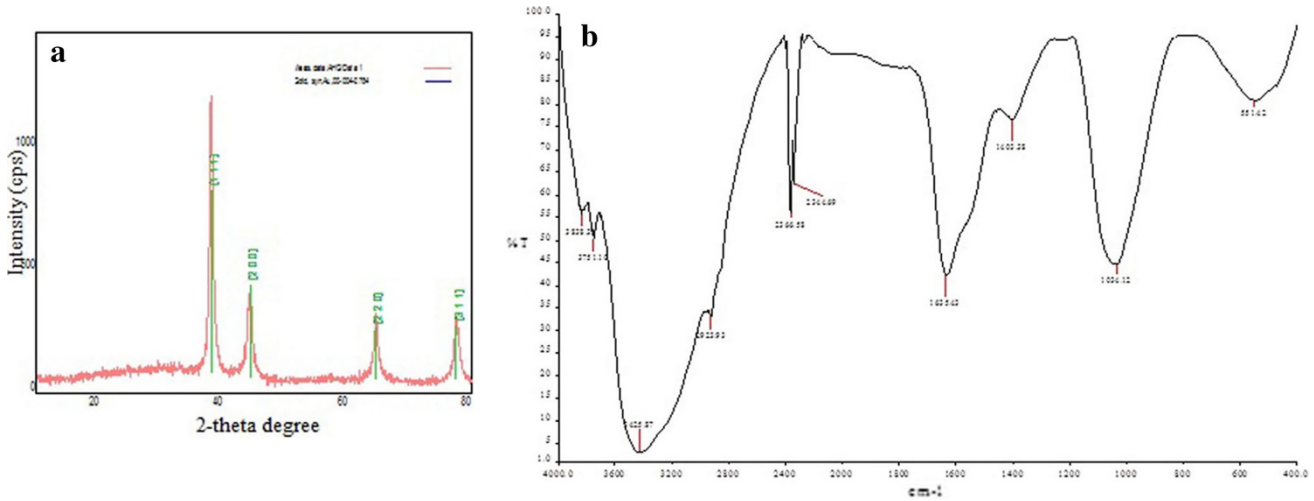

Fig. 3 **a** XRD and **b** FTIR results of GNPs synthesised in *Chlorella vulgaris* extract

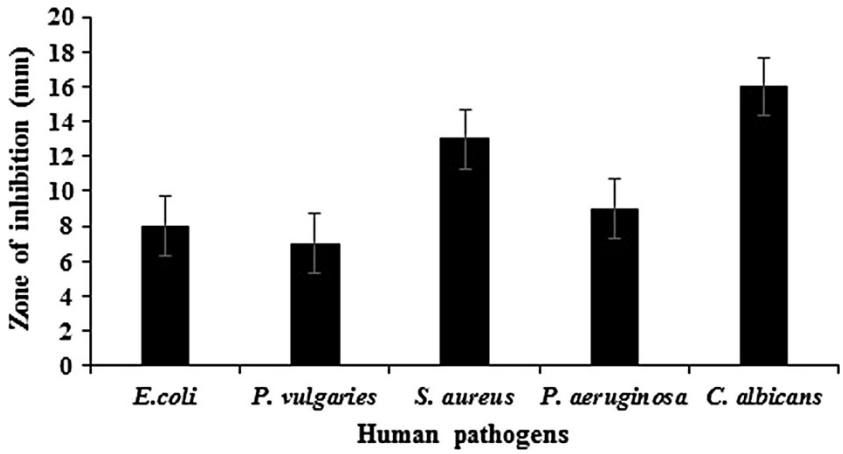

Fig. 4 Graph representing the zone of inhibition by GNPs against human pathogens

engaged in the dual function of Au III reduction and healthy capping of the GNPs. The positively charged groups of the *C. vulgaris* biomolecules might have regulated the surface-mediated process by means of 'electrostatic interaction', which could have begun with the nucleation of Au^0 to Au atoms and could have finally formed into GNPs.

Anti-pathogenic assay

GNPs were found to be toxic to human pathogens (*E. coli*, *P. vulgaris*, *S. aureus*, *P. aeruginosa* and *C. albicans*) and exhibited the maximum inhibition against *C. albicans* of zone 16 mm and *S. aureus* with 14 mm. Other three human pathogens were moderately susceptible (Fig. 4).

The capability of the *C. vulgaris* phytochemicals to efficiently reduce the chloroaurate ions into biocompatible GNPs and its toxicity to human pathogens has thus been demonstrated. This single-step green method reveals *C. vulgaris* extract as a competent resource for both the manufacturing and nontoxic biomimetic capping of GNPs and application in the medicinal field.

Conclusion

Biosynthesis of GNPs using aqueous extract of *C. vulgaris* is a safe and self-sufficient electron donor system during the bioreductive precipitation of gold without any external chemical reagent. Thus, no toxic by-product formation occurs during the synthesis of GNPs via, 'green chemistry' and biomolecules in *C. vulgaris* establishes a rapid reduction process for the conversion of gold ions into GNPs. This type of process will play an important role in nanoparticle synthesis and their application in drug development and biomedical tool designing in future.

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