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Short communication

Aqueous extract of gum olibanum (*Boswellia serrata*): A reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles

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ABSTRACT

A simple and ecofriendly biosynthetic process has been developed for silver nanoparticles using the aqueous extract of gum olibanum (*Boswellia serrata*), a renewable natural plant biopolymer. The water soluble compounds in the gum serve as dual functional reducing and stabilizing agents. The effect of concentration of gum and silver nitrate; and reaction time on nanoparticle synthesis was studied. The UV–visible spectroscopy, transmission electron microscopy and X-ray diffraction techniques were used to characterize the synthesized nanoparticles. By tuning the reaction conditions, size controlled spherical nanoparticles of around 7.5 ± 3.8 nm was achieved. Using Fourier transform infrared spectroscopy and Raman spectroscopy, a probable mechanism involved in reduction and stabilization of nanoparticles has been explained. The produced silver nanoparticles exhibited substantial antibacterial activity on both the Gram classes of bacteria. By virtue of being biogenic and encapsulated with proteins, these surface functionalized nanoparticles can be easily integrated for various biological applications.

1. Introduction

The plant based exudate gums such as gum *Acacia* [1] and gum kondagogu [2,3]; microbial polysaccharides; gum gellan [4] and carboxymethylated-curdlan [5] and marine polysaccharide gums including fucoidan [5] and alginate [6] have been utilized as reducing and stabilizing agents for silver, gold and platinum nanoparticle biosynthesis. The nanoparticles stabilized by natural polymers make them nontoxic to cells and render them as suitable candidates for safe delivery of drugs, molecular imaging and biomedical diagnostics. Biocompatibility and antibacterial activity are the prerequisites for using silver nanoparticles as coating materials in biomedical engineering and food packaging. Both these properties were reported wherein silver nanoparticles were stabilized with chitosan [7]. Besides, the preparation of silver nanoparticles with hydroxylated water soluble polymers facilitates the control of particle mean diameter and size distribution [1,2]. In this context, we have explored the use of gum olibanum for biosynthesis of silver nanoparticles.

Gum olibanum is a naturally occurring gum-oleo-resin derived as an exudate from the bark of *Boswellia serrata* (Burseraceae family), a native tree of India. Besides its use as incense, fumigant and multipurpose aromatic; it is also exploited in food, pharmaceutical, paint, ceramic, cosmetic and textile industries. It posses a broad spectrum of properties and has long been used in the traditional Ayurvedic and Unani medicines [8,9]. Typically the gum consists of volatile oil, water soluble gum (polysaccharides), lipophilic terpenes and insoluble matter. The polysaccharide is abundant in neutral sugars and composed of galactose, arabinose, xylose and D-glucuronic acid [8,10]. The water-soluble polysaccharide fraction was employed as a potent vaccine adjuvant [11], matrix for controlled release of diclofenac [12], hypolipidemic, hepato-protective, reno-protective [13] and anticancer agent [14]. The toxicity and safety studies have established that the gum is non-toxic and safe for use in different animals [15,16].

The natural availability, non-toxic nature, low cost and medicinal values of gum olibanum intrigued us to use this biopolymer for the silver nanoparticle synthesis. In this context, we have designed a facile, biosynthetic route for the production of silver nanoparticles employing a renewable, biodegradable natural plant polymer, gum olibanum as both the reducing and stabilizing agent. The intend of the present study was on the synthesis, characterization, capping and stabilization of silver nanoparticles. We have also shown the antibacterial activity of the prepared nanoparticles on Gram-positive and Gram-negative bacteria for prospective biological applications.

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2. Materials and methods

2.1. Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) (E. Merck, Mumbai, India) and "Gum olibanum", grade-1 (Girijan Co-operative Corporation Ltd., Hyderabad, India) were used for the synthesis. The gum was powdered and sieved to obtain a mean particle size of 38 μm. Then 0.5% (w/v) of homogenous gum stock was prepared by stirring the solution overnight and the obtained supernatant after centrifugation was used for all the experiments. The silver nanoparticles were synthesized by autoclaving the $AgNO_3$ solutions containing various concentrations of gum at 121 °C and 103 kPa of pressure for different durations of time. The effect of concentration of gum and $AgNO_3$; and reaction time on nanoparticle synthesis was studied.

2.2. Characterization of synthesized silver nanoparticles

The synthesized nanoparticles were characterized by UV–visible spectroscopy (Elico SL 196, Hyderabad, India), transmission electron microscopy (JEOL 3010, Tokyo, Japan), X-ray diffraction (Rigaku Ultima IV, Tokyo, Japan), Fourier transform infrared spectroscopy (Bruker Optics Tensor 27, Ettlingen, Germany) and Raman spectroscopy (Suwtech G-SLM Diode Laser, Shanghai, China). SDS-polyacrylamide gel electrophoresis of the gum before and after autoclaving with $AgNO_3$ was performed with stacking and separating gels (5 and 15%, w/v) based on the method of Laemmli.

2.3. Antibacterial assay

The well diffusion method was used to study the antibacterial activity of the synthesized silver nanoparticles. *Staphylococcus aureus* (ATCC 25923); and *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as model test strains for Gram-positive and Gram-negative bacteria, respectively. The nanoparticles used here were prepared with 0.5% gum solution containing 1 mM $AgNO_3$, autoclaved for 30 min (Supplementary data).

3. Results and discussion

3.1. Characterization of synthesized silver nanoparticles

3.1.1. UV–visible (UV–vis) spectroscopy

The formation of nanoparticles was recorded by measuring the absorption spectra of synthesized silver nanoparticles against respective autoclaved gum blanks. As shown in Fig. S1 (Supplementary data), the photograph is indicating (a) gum tears of grade 1 quality, (b) gum powder sieved to 38 μm particle size and (c) centrifuged gum solution of 0.5%. The role of gum concentration on the synthesis was studied by autoclaving different concentrations (0.1–0.5%) of gum solutions containing 1 mM of $AgNO_3$ for 30 min (Fig. 1(a)). After autoclaving, the appearance of yellow color in the reaction mixtures was observed, a clear indication of silver nanoparticle formation by the gum. It reveals that with increasing in gum concentration, there is an enhancement in the nanoparticle concentration. Further, the nanoparticle production with 0.5% gum was monitored by varying $AgNO_3$ concentration for 30 min of autoclaving (Fig. 1(b)). Upon increasing the concentration of metal precursor, the intensity of the absorption band increased, due to an enhancement in the nuclei formation, results in the production of larger number of nanoparticles. The synthesis was also evaluated by varying the reaction time (10–60 min) and reduction was studied with 0.5% gum at 1 mM $AgNO_3$ (Fig. 1(c)). It was noticed that the intensity of the absorption increased with time, indicating a continuous reduction of the silver ions, leading to the increased number of nanoparticles. In these UV–vis spectra a single strong peak with a maximum around 418 nm was observed, corresponds to the typical surface plasmon resonance of conducting electrons from the surface of silver nanoparticles. SDS-PAGE of the gum was carried out before and after autoclaving to find out the fate of the proteins associated with the gum. Electrophoretic analysis of the gum indicated the presence of four protein bands with an apparent molecular weight of 59, 54, 50 and 42 kDa. These proteins were found to be intact and stable, even after autoclaving the gum solution with and without $AgNO_3$ (data not shown).

3.1.2. Transmission electron microscopy

Figs. S2 and S3 show the TEM images of the nanoparticles synthesized with 0.1% gum and 1 mM $AgNO_3$, autoclaved for 30 and 60 min, respectively. The population is dominated by spherical nanoparticles in varying sizes and also a small number of quasi spherical and uneven shaped nanoparticles were observed. These nanoparticles are polydisperse and the average particle sizes were about 32.1 ± 12.5 and 22.4 ± 10.2 nm, respectively, for 30 and 60 min of autoclaving. The influence of gum concentration on nanoparticle size was investigated with 0.5% gum and 1 mM $AgNO_3$, autoclaved for 30 min (Fig. 2). These nanoparticles are spherical in shape and the average particle size was about 7.5 ± 3.8 nm (Fig. 2(e)). When the concentration of gum was increased from 0.1 to 0.5%, the average size of the nanoparticles formed decreased [1,2,6]. The decrease in polydispersity and mean particle size with increase in gum concentration was also evident from the TEM images; and the decreased full width at half maximum (FWHM) values of respective UV–vis peaks, from 85 nm (0.1% gum) to 75 nm (0.5% gum) (Fig. 1(a)). This may be due to the contribution of further reduction as well as intense stabilization process between capping molecules and surfaces of nanoparticles. Particularly, nearly 50% of the nanoparticles formed were in the range of 2.4–3.6 nm (Fig. 2(e)). This study indicates that the overall size of the nanoparticles can be controlled by varying the concentration of gum and reaction time [2].

3.1.3. X-ray diffraction (XRD)

There were five well-defined characteristic diffraction peaks at 38.3°, 44.5°, 64.7°, 77.6° and 81.8°, respectively, corresponding to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes of face centered cubic (fcc) crystal structure of metallic silver (Fig. S4). The interplanar spacing values (d_{hkl}) values (2.348, 2.034, 1.439, 1.229 and 1.176 Å) and the lattice constant (4.065 Å) calculated from the XRD spectrum of silver nanoparticles are in agreement with the standard silver values (JCPDS PDF card 04-0783). Thus, the XRD pattern, further corroborate the highly crystalline nature of nanoparticles observed from SAED pattern and high-resolution TEM image (Fig. 2). It is clear that for the synthesized silver nanoparticles the (1 1 1) lattice plane is the preferred orientation [2], which is also known for their high antibacterial activity [17] (Fig. S4).

3.1.4. Fourier transform infrared (FTIR) spectroscopy

The major absorbance bands present in the spectrum of gum were at 3395, 2963, 2926, 2858, 2145, 1651, 1605, 1520, 1420, 1383, 1261, 1093 and 1022 cm^{-1}, respectively (Fig. 3). The broad bands observed at 3350 and 2145 cm^{-1} could be assigned to respective stretching vibrations of O—H and various carbonyl groups of the gum. The bands at 2963, 2926, and 2858 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of methylene groups. The bands found at 1605; and 1420 and 1383 cm^{-1} could be assigned to characteristic asymmetrical and symmetrical stretches of carboxylate group. The observed bands at 1651 and 1520 cm^{-1} can be identified as amide I and amide II linkages of the proteins. The sharp peak at 1261 and peaks at 1093 and 1022 cm^{-1} correspond to C—O stretch of carboxylic acids; and ether and alcoholic groups. For lyophilized nanoparticles, the characteristic absorbance bands were noted at 3435, 2963, 2925, 2855, 2014, 1630, 1537, 1454, 1398, 1261, 1093 and 1026 cm^{-1}, respectively. In the case of nanoparticles, a large shift in the absorbance peak with decreased band intensity was observed from 3395 to 3436 cm^{-1} and 1420 to 1454 cm^{-1}, implying the binding of silver ions with hydroxyl and carboxylate groups of the gum [1]. It is pertinent to note that the nanoparticles show a large band shift corresponding to carbonyl groups (2145–2014 cm^{-1}) and also, the band intensity of carboxylic acid groups was found to be decreased for nanoparticles. Based on these band shifts, it can be concluded that both hydroxyl