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Activity study of biogenic spherical silver nanoparticles towards microbes and oxidants

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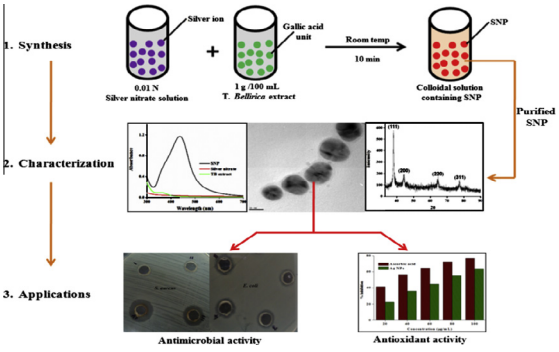
HIGHLIGHTS

- *T. bellirica* fruit extract mediated synthesis of spherical SNP.
- Identification of secondary metabolites in fruit extract by HPLC.
- Provided plausible mechanism involved in the formation of stable SNP.
- Evaluation of antimicrobial and antioxidant activity with plausible mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

The eco-friendly approach for the green synthesis of silver nanoparticles (SNP) using *Terminalia bellirica* (*T. bellirica*) fruit extract is reported herein. Initially formation of SNP was noticed through visual color change from yellow to reddish brown and further analyzed by surface plasmonic resonance (SPR) band at 429 nm using UV–Vis spectroscopy. Identification of different polyphenols present in *T. bellirica* extract was done using High Pressure Liquid Chromatography (HPLC). Aqueous *T. bellirica* extract contains high amount of gallic acid which is major secondary metabolite responsible for the reduction and stabilization process. It was established by analyses of extracts before and after reduction using HPLC. Formation of spherical SNP was characterized by Transmission Electron Microscopy (TEM) analysis. X-ray Diffraction (XRD) study revealed crystalline nature of SNP. Presence of different functional groups on the surface of SNP was evidenced by Fourier Transform Infrared Spectroscopy (FTIR) study. A plausible mechanism of reduction and stabilization processes involved in the synthesis of stable SNP was also explained based on HPLC and FTIR data. In addition, the synthesized SNP was tested for antibacterial and antioxidant activities. SNP showed good antimicrobial activity against both gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. It also showed good antioxidant activity compared to ascorbic acid as standard antioxidant by using standard DPPH method.

Introduction

Nanomaterials has become the most important and powerful tool for mankind in the present generation leading to new inventions because of their inimitable and superior properties such as electrical, optical, magnetic and catalytic, which are absent in bulk material [1]. Synthesis of noble metal nanoparticles (NP) has been widely employed by the researchers because of their improved physicochemical properties [2] leading to enormous applications in various fields. Among them silver nanoparticles

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(SNP) has gained lot of impact because of their applications in interdisciplinary fields such as medicine, biosensors and electronics [3]. Also SNP shows tough toxicity towards wide range of microorganisms and hence they have been used in textiles, water cleaners, room sprays and food storage containers etc. [4]. Many physical and chemical methods are available in synthesizing SNPs which includes laser ablation, thermal decomposition, microwave, sonoelectrochemical and chemical reduction, [5–8]. Whereas the synthesis of SNP by above mentioned methods have the following drawbacks. Physical methods are very expensive, time consuming, needs high energy and sophisticated instruments; on the other hand chemical methods involves the use of harsh chemicals for the synthesis and results in the formation of abusive by-products which leads to the contamination of NPs [9]. Since SNPs have been used in optical, electrical and human contacting applications [10], it is necessary to move towards non-toxic and inexpensive eco friendly actions for green and safer NP fabrication. To accomplish the demand of toxic free NPs majority scientists have preferred green synthesis of SNP using different prokaryotic and eukaryotic organisms. Biogenic synthesis of SNP using bacteria [11], yeast [12], fungi [13] and plants [14] as reducing and stabilizing agents have been widely practiced from past decade as an best alternative to both chemical and physical conventional methods. Among these green reducing agents plant extracts has been executed widely to synthesize NP because of their simplicity, inexpensive and eco-friendly nature and also avoids the lengthy procedure of maintaining cultures and monitoring reactions. Decent literatures are available on the plant mediated synthesis of SNP [14]. Recently different plant species such as *Artocarpus heterophyllus* [15], *Brucea javanica* [16], *Dillenia indica* [17], *Mimusops elengi* [18], *Punica granatum* [19], and *Sesbania grandiflora* [20] have been used in the synthesis of SNP. Though lot of works has been explored in this area still this diversified field is gaining a lot of attention in order to understand the potential of plant extracts as both reducing and stabilizing agent also the chemistry involved in enhanced stability and improved biological activity of synthesized NP. Conversion of the metal salts to their nano form using plant extracts mainly depends on the availability of the polyphenols in the extract and identification of these components aids researchers to understand the possible mechanism in the reduction and stabilization processes. *Terminalia bellirica* (*T. bellirica*) is a large deciduous tree common on lower hills in Southeast Asia and also grown as an avenue tree. *T. bellirica* belongs to Combretaceae family which has been widely used as Ayurvedic medicine and also one of the constituent in well recognized herbal medicine Triphala (traditional medicine containing three different fruits *Emblica officinalis, Terminalia chebula* and *T. bellirica*) for healing skin diseases, cough, diarrhoea and oral thrush [21]. Present study reports the synthesis of spherical SNP by *T. bellirica* fruit (pericarp) extract and identification of the secondary metabolites present in the plant extract responsible for reduction/stabilization by using HPLC. Further synthesized SNPs were characterized using different instrumental techniques, tested for antimicrobial and antioxidant activities. Finally, the present manuscript has proposed plausible mechanisms for stabilization of nanoparticles by plant secondary metabolites as well as their antimicrobial and antioxidant activities.

Materials and methods

Preparation of T. bellirica fruit extract

T. bellirica fruits were collected from the local market Vellore, Tamil Nadu, India. Fruit pericarp was separated, dried and finely ground. Prior to experiment the dried powder was kept in oven for 20 min at 60 °C to remove the moisture, then 0.03 g was taken in a beaker containing 100 mL of deionized water and warmed in the temperature controlled water bath at 90 °C for 30 min and the obtained extract was filtered using 0.22 μm cellulose nitrate membrane filter paper for further studies.

Synthesis of silver nanoparticles

Aqueous silver nitrate (10 mL of 0.01 M) was added to 100 mL of freshly prepared extract and stirred thoroughly at ambient condition. Appearance of brown color indicated the formation of SNP which was further confirmed by UV–Vis spectroscopy after sampling the reaction mixture at different time intervals.

Characterization

Initially formation of SNP was monitored after 10 folds dilution of the colloidal solution with deionized water and spectra were recorded between 300 nm and 700 nm using Jasco V-670 UV–Visible double beam spectrophotometer. Then colloidal solution was centrifuged at 12,000 rpm and the pellet obtained was washed thoroughly with deionized water for 3 times; dried and used for further microscopic and spectroscopic characterization. The morphology and size of the bioreduced SNP was visualized by placing a drop of sonicated well dispersed sample on Cu grid using JEOL JEM 2100 HR-TEM at an acceleration voltage of 200 kV with a resolution of 0.1 nm. For XRD studies purified SNP were coated on XRD grid and spectra were recorded in 2θ region from 10° to 90° with a scanning rate of 4°/min and with a step size increase of 0.02° using Bruker D8 Advance Diffractometer with Cu Kα radiation (λ = 1.54 Å). In order to determine the functional groups on the surface SNP were pelletized with KBr and then spectra were recorded using JASCO FT-IR 4100 instrument in the diffuse transmittance mode at a resolution of 4 cm^{-1}. For comparison, spectra of *T. bellirica* fruit powder were recorded. To know the possible water soluble phyto-constituents present in fruit extracts HPLC analysis of fruit extract was done using Perkin Elmer 200 Series HPLC equipped with UV–Vis detector (192–700 nm) and a 200 Series pump. The sample was eluted using a mobile phase containing 0.1 M KCl and 32% acetonitrile (pH was adjusted to 3.0 with dil. HCl) using Brownlee Analytical C-18 (150 × 4.6 mm 5 μm 110 Å) column packed with 5 μm silica particles. The detection was carried out using UV–Visible detector at 260 nm with a flow rate of 1 mL min^{-1}. The peaks that obtained were compared and matched with external standards.

Antibacterial activity

The synthesized SNP were tested against gram-positive bacteria *Escherichia coli* (*E. coli*) (strain ATCC 25922) and gram negative bacteria *Staphylococcus aureus* (*S. aureus*) (strain ATCC 25923). Agar well diffusion method was used in order to determine toxicity of synthesized SNP (purified and redispersed in water) towards both bacterial strains. Muller Hinton Broth (MHB) containing 1% Agar was used to prepare medium for easy diffusion of NP. Bacterial lawn was prepared on sterile MHB plates by using sterile cotton swabs and approximately 8 mm wells were made on the nutrient medium. Different concentrations of SNP ranging from 10–100 μg/mL (20 50, 75 and 100 μg/mL) were placed in the specified wells, and then plates were incubated at 37 °C overnight. The activity against both the bacterial cultures was determined by measuring the zone of inhibition (ZOI) and found out minimum inhibitory concentration (MIC). Standard Amoxicillin disc (10 mg) was used as positive control and plant extract before reduction of SNP was also used for the comparison.