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Research Article

Bombax ceiba thorn extract mediated synthesis of silver nanoparticles: Evaluation of anti - *Staphylococcus aureus* activity

Roshan Telrandhe [1]*, Debarshi Kar Mahapatra [2], Manish A. Kamble [1]

[1] Department of Pharmacognosy, Kamla Nehru College of Pharmacy, Nagpur 441108, Maharashtra, India
[2] Department of Pharmaceutical Chemistry, School of Pharmacy, Swami Ramanand Teertha Marathwada University, Nanded 431606, Maharashtra, India

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Abstract

The current research aimed at fabricating plant extract mediated biosynthesized silver nanoparticles (AgNPs) utilizing thorn extract of *Bombax ceiba* (TEBC). The synthesized AgNPs was characterized by UV spectroscopy where the surface plasmonic resonance peak (SPR) was located at 222 nm. The scanning electron microscopy (SEM) studies demonstrated that the morphology of fabricated nanomaterials was primarily cylindrical of average size of 20-30 nm with some spindles of size >50 nm. The anti-microbial evaluation against *Staphylococcus aureus* revealed that AgNPs exhibited notable activity with ZOI of 27.2 mm at MIC of 25 μg/mL. The outcome of this research evidently signified that the biofabricated AgNPs using TEBC may be a new greener approach or technology to formulate anti-bacterial nanodrugs in future.

Keywords: *Bombax ceiba*, extract, silver, nanoparticle, biosynthesis, green synthesis.

INTRODUCTION

Nanotechnology remains the most promising, emerging, and interesting section of research in 21st century owing to its diverse applications with modified physico-chemical attributes as compared to the bulk material[1]. In the last few decades, the application of nanomaterials in the area of biology and medicine has revolutionized the field of drug delivery, theranostics, imaging, diagnosis, wound healing, and medical devices with miscellaneous properties[2]. Nanomedicine is the sub-branch expanding where nanocomposite products and technologies of 1-100 nm range are of great importance.

Metallic nanoparticles are foremost preferred in material chemistry owing to their physicochemical characteristics. Silver nanoparticles have citadel importance in the present era and are extensively used in pharmaceutical sciences, drug delivery, biomedical sciences, catalysis, etc[3]. They can be conjugated with various functional biomolecules and can be used to target numerous diseases. The role of silver as anti-bacterials is known for centuries and is seen in museums as daily use products where they are majorly composed[4]. These nanomaterials possess high ratio of surface area to mass and have amazing bactericidal properties by diverse mechanism of actions[5]. The anti-microbial activity is majorly believed to be the cumulative toxic effect of the product which is exerted by metallic nature causing detrimental effects on normal cellular proliferation and protein functions[6].

Since late 90s, the silver nanoparticles were synthesized by chemical, physical, or photochemical methods[7]. As cost, particle size and distribution, scalability, and dispersion remained the crucial aspects, chemical method for the nanoparticle fabrication was usually applied[8]. However, in the due course of time, the adverse toxic effect to the environment compelled the producers to opt for safer, environment friendly, green technique for the fabrication[9]. In recent times, this approach received substantial notice owing to lowest production cost, safety, environmentally friendly aspects, and simplest procedure for the synthesis. The plant biomass serves as reductant and capping agents, where an aqueous solution of metallic salt reacts to form nanoparticles rapidly[10].

Bombax ceiba or silk cotton tree, belonging to the family of Bombacaceae is a large deciduous tree found throughout India. It is well known for its medicinal properties ethnopharmacologically in the society for centuries and in traditional systems like Ayurveda, Siddha, and Unani. The plant extract is known for pharmacological activities such as anti-inflammatory, anti-hypertensive, anti-microbial, analgesic, anti-angiogenic, anti-viral, etc[11]. Recently, *Bombax ceiba* leaf extract has been employed to synthesize silver nanoparticles. The thorn extract of *Bombax ceiba* (TEBC) is reported to have potent anti-oxidant, anti-microbial, anti-inflammatory activities[12]. However, there is no report of exploiting the thorn extract to fabricate size controlled and uniform silver nanoparticles. The present research aimed at synthesizing silver nanoparticles utilizing TEBC as a green approach to provide the foundation for commercial production of nanomaterial with environmental friendly attributes.

MATERIALS AND METHODS

Chemicals

Silver nitrate was purchased Sigma-Aldrich (Germany). Other analytical grade reagents and consumables were procured from HiMedia (India). Double distilled water apparatus (Borosil®, India) was used for the experiment.

Collection

The fruits of *Bombax ceiba* were collected from the tree present at the medicinal garden campus of Kamla Nehru College of Pharmacy situated in the Butibori area of Nagpur City in Maharashtra State of India. The plant was identified and authenticated by Dr. Dongarwar, Department of Botany, RTM Nagpur University, Nagpur, Maharashtra.

Extraction

The thorns were collected from the tree, dried in the shade for a specified period, and powdered suitably. The dried powder, divided into multiple smaller amounts, was subjected to continuous hot Soxhlet extraction with 50 mL distilled water and 50 mL alcohol (ethanol 90%) in equal ratio at a temperature of 55-65°C during 32 cycles. The solvent was removed under reduced pressure and controlled temperature using a rotary vacuum evaporator. The hydroalcoholic thorn extract of *Bombax ceiba* (TEBC) yield was found to be 11.8% w/w.

Preparation of silver nitrate solution

For the experiment, 1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared by dissolving 0.01698g of $AgNO_3$ in 100 mL of double distilled water.

Synthesis of silver nanoparticles

For the preparation of silver nanoparticles, 1 mL of TEBC was taken and in that 9 mL of 1mM aqueous solution of silver nitrate was added. The content was incubated at 37°C for 24 hours. The color change was checked frequently, which is an indicator of silver nanoparticles formation[13].

Characterization of silver nanoparticles

UV-Vis spectroscopic study

The silver nitrate solution and reduction of pure silver ions was monitored after 24 hours on a UV-Vis spectrophotometer (Shimadzu® UV-1800, Kyoto double beam model) in 200-800 nm wavelength range. The samples were prepared in small aliquots with distilled water[14].

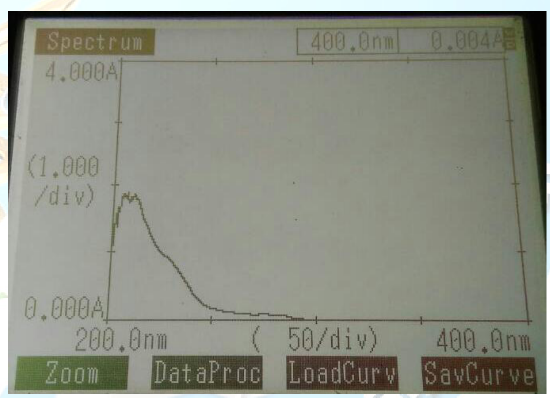

Figure 1. UV spectra of synthesized nanoparticles.

Scanning electron microscopic study

The morphology of the fabricated silver nanoparticles was studied using scanning electron microscopy (SEM) technique (JOEL-JSM 6390 SEM machine). For the analysis, the samples were prepared by sprinkling the nanomaterial on the double tape wedged to the aluminium stub. Subsequently, the sample containing stub was then placed in the

SEM chamber and scanned randomly at the acceleration voltage of 10 kV. The photomicrographs were taken and the results were recorded[15].

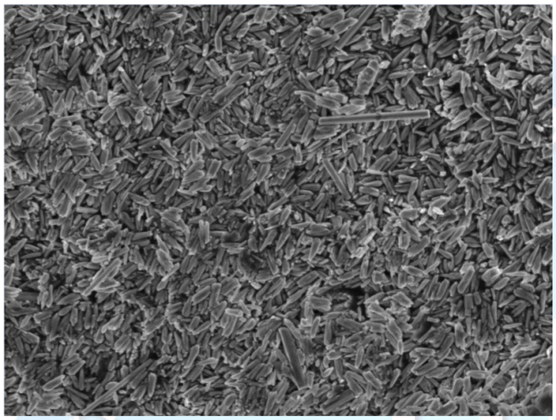

Figure 2. SEM micropictograph of fabricated silver nanoparticles.

Anti-bacterial activity

The *in-vitro* anti-bacterial activity of silver nanoparticles was performed by disc diffusion method in a triplicate manner using Muller Hinton Agar medium against a pathogenic bacterial strain *Staphylococcus aureus* (*S. aureus*, MTCC 3160). *S. aureus* was initially cultured in nutrient broth and incubated at 37°C for 24 hr and then the cultured cells were tend to multiply in the Muller Hinton agar plates. Afterward, the silver nanoparticle containing discs were placed over the bacterial plates and incubated at 37°C for 24 hrs, comparing ciprofloxacin as the positive control). The diameter of the zone of inhibition (ZOI) was measured in millimeters (mm).

The minimum inhibitory concentration (MIC) is the smallest concentration in which the compound displays no visible microbial growth. It was determined by agar streak dilution method in triplicate manner. The protocol involve formation of microbial suspension (~10^5 CFU/mL), application to the petridish with serial dilution and incubation of petridish at 37±1ºC. The MIC value was determined and the average was taken[16].

RESULTS AND DISCUSSION

It was observed that upon addition of the extract into the flask containing the aqueous silver nitrate solution, the color of the medium changed to dark brown within 2 min. This reflected the conversion of ionic form of silver into the elemental form. The formation of the silver nanoparticles was further confirmed by two important analytical techniques: UV spectroscopy and scanning electron microscopy.

UV spectroscopic study

The nanoparticle sample displayed the absorbance peak of about 222 nm. The peaks arise due to the absorption phenomenon of metallic silver nanoparticles as a result of the Surface Plasmon Resonance (SPR). The absorption peak near to 200 nm represents the interaction of numerous organic molecules with the silver ions in the solution whereas the disturbances observed at nearly 300 nm probably indicated that capping occured after the reduction of the silver nanoparticles. The above occurrence suggested the plausible mechanism(s) for the reduction of the silver ions into their elemental form. **Figure 1** describes the UV spectra recorded from the aqueous solution of silver nitrate with TEBC.

Scanning electron microscopic study

The morphology of the fabricated nanoparticles was identified to be primarily cylindrical. A few nanoparticles were rod shaped and long. However, no round or circular geometry was observed. Although, the disparity in terms of size, shape, morphology, etc. are usually seen in nanomaterials synthesized by plant extracts. It was noticed that the nanoparticles edges were much lighter than that of the centre which indicated that the proteins capped the silver nanoparticles. The SEM images demonstrated average size of about 20-30 nm with some spindles of size >50 nm (**Figure 2**).

Anti-microbial activity

The silver nanoparticles exhibited fairly good anti -*S. aureus* activity as compared to the standard drug ciprofloxacin. The nanomaterial exhibited an impressive ZOI of 27.2 mm at MIC of 25 μg/mL whereas ciprofloxacin exhibited 32.6 mm ZOI at MIC of 6.25 μg/mL. Therefore, it may be concluded that the fabricated nanomaterial have potential to exhibit antimicrobial activity.

CONCLUSION

The research concluded that TEBC demonstrated the biofabrication of AgNPs of average size 20-30 nm. The characterization of AgNPs by sophisticated analytical tools showed that the nanomaterials were formed as desired. The anti-microbial evaluation against *Staphylococcus aureus* revealed that AgNPs exhibited notable activity with ZOI of 27.2 mm at MIC of 25 μg/mL. The outcome of this research evidently signified that the biofabricated AgNPs using TEBC may be a new greener approach or technology to formulate anti-bacterial nanodrugs in future.

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