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One pot green fabrication of metallic silver nanoscale materials using *Crescentia cujete* L. and assessment of their bactericidal activity

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Abstract: In this study, the leaf extract of an important medicinal plant *Crescentia cujete* L. (CC) was employed as a green reducing agent to synthesise highly-stable *C. cujete* silver nanoparticles (CCAgNPs). The reduction of Ag^+ to Ag^0 nanoparticles was initially observed by a colour change which generates an intense surface plasmon resonance peak at 417 nm using a UV-Vis spectrophotometer. Various optimisation factors such as temperature, pH, time and the stoichiometric proportion of the reaction mixture were performed, which influence the size, dispersity and synthesis rate of CCAgNPs. In addition, surface chemistry of synthesised CCAgNPs through Fourier transform infrared spectroscopy reveals the reducing/stabilising agent present in the aqueous extract of *C. cujete* and synthesised CCAgNPs. Transmission electron microscopy analysis features the spherical shape of CCAgNPs with an average size of 39.74 nm. Furthermore, an X-ray diffraction study confirms that the synthesised CCAgNPs were face-centred cubic crystalline in nature. The CCAgNPs display tremendous bactericidal activity against human pathogens *Bacillus subtilis*, *Staphylococcus epidermidis*, *Rhodococcus rhodochrous*, *Salmonella typhi*, *Mycobacterium smegmatis*, *Shigella flexneri* and *Vibrio cholerae* via penetrating into the bacterial cell membrane and causing failure of an internal chain reaction.

1 Introduction

Recently, researchers around the globe were drawn to search for new biofunctionalised nanomaterials for enhanced therapeutic applications. Interestingly, nano-scaled materials have drawn the attention of research fraternity due to their specific properties such as the large surface area to volume ratio, tuneable surface, high stability and durability [1]. Nanoparticles ranging from 1 to 100 nm exhibit new physiochemical, optoelectronic and biological properties than their corresponding bulk materials [2]. Based on size, shape and morphology/topological characteristic features of nanomaterials, nanoparticles were rapidly gaining renewed attention in healthcare, pharmaceutical field, electronics, chemical industries, food industries, and environmental management [3].

Metal nanoparticles such as silver, gold, copper, platinum, and palladium were the choice of most research due to their tremendous nanobiotechnological applications. Among the metal nanoparticles, silver nanoparticles (AgNPs) ranked top in order as it holds astonishing physio-chemical features and broad biotechnological applications [4]. Conventional synthesis protocols (physical and chemical) for fabricating AgNPs were found to be expensive, which requires high energy consumption, utilisation of hazardous chemicals, toxicity and difficulty in purification [5]. To overcome these obstacles, a new alternative route through the nanobiotechnological approach has to be developed for sustainable growth.

Currently, several multi- and unicellular organisms were employed to fabricate metallic nanoparticles. Among them, plant remains as the better choice for metal nanoparticles synthesis than microbes due to their availability, cost-effective, speedy synthesis and easy downstream processing [6]. It was a well-known fact that plant extracts contain active secondary metabolites such as terpenoids, flavonoids, alkaloids, tannins, saponins and phenols which can act as a reducing/stabilising agent to modulate ionic metal salts into metallic nanoparticles [7]. Optimisation of reaction parameters was very important to obtain nanoparticles with narrow size distribution [8].

Hitherto, various plant entities have been explored for AgNPs' synthesis the availability of plant species and their prospects as nanoformulations still continues. Originally, *C. cujete* L. (Calabash tree) was native to central and south America, but later widespread throughout the world. It possesses various biological properties and practiced in traditional and folk medicine [9, 10]. Henceforth, the present study aimed to fabricate AgNPs using an aqueous leaf extract of *C. cujete* L. and further to evaluate its antimicrobial efficacy against human pathogens.

2 Materials and methods

2.1 Materials

All the media components and analytical reagents were purchased from Hi-Media Laboratories Pvt Ltd (Mumbai, India) and Sigma Aldrich Chemicals (St. Louis, USA).

2.2 Preparation of extract

For the preparation of extract, fresh and healthy leaves of *C. cujete* L. were collected from Bharathidasan University campus, Tiruchirappalli, Tamilnadu, India. The collected plant leaves were washed thoroughly thrice with distilled water to remove contaminants and shade dried for 10 days. After drying, the leaves were grounded finely into powder using a kitchen blender. The *C. cujete* extract was prepared by mixing 10 g of powder with 100 ml of distilled water and kept in boiling water bath at 60°C for 20 min. Furthermore, the extract was filtered through Whatman filter paper No. 1 to remove plant debris. Finally, the filtered extract was stored in a refrigerator at 4°C for further use.