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Antimicrobial efficacy of green synthesized drug blended silver nanoparticles against dental caries and periodontal disease causing microorganisms

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ABSTRACT

Development of biologically inspired green synthesis of silver nanoparticles is evolving into an important branch of nano-biotechnology. In the present investigation, we report the green synthesis of silver nanoparticles (AgNPs) employing the leaf extract of *Justicia glauca*. Water-soluble organics present in the leaf extract are mainly responsible for the reduction of silver nitrate ($AgNO_3$) solution to AgNPs. The AgNPs are 10–20 nm in dimensions as determined by TEM images. The antimicrobial activities of green synthesized AgNPs and drug blended AgNPs have been evaluated against the dental caries and periodontal disease causing microorganisms such as *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The AgNPs and drug blended AgNPs show a significant antibacterial and antifungal activity. Minimum inhibitory concentration (MIC) value of AgNPs determined against the selected dental caries and periodontal disease causing microorganisms are noticeable between the range of 25–75 μg/mL.

1. Introduction

In the recent past, there has been a remarkable attention in the field of nanotechnology to develop reliable and promising process for the synthesis and stabilization of metal nanoparticles, especially in noble metals such as silver, gold, and platinum owing to their prospective applications in the fields like optical devices, drug delivery systems and biological labeling [1,2]. For the past two decades, numerous methodologies have been formulated to synthesize metal nanoparticles with different sizes, shapes and controlled surface morphologies [2]. Usually metal nanoparticles synthesis is carried out by various physical and chemical methods like laser ablation, lithography, chemical vapor deposition, sol–gel technique and electro-deposition, which are very expensive and highly toxic [3,4]. Therefore, bio-material scientists are looking forward to develop clean, nontoxic chemicals, environmentally benign solvents and renewable materials for the synthesis of metal nanoparticles. As a result, various microbes and plant have been employed to synthesize the nanoparticles via green-approach [5,6]. During green approach, the extract from bio-organisms releases variety of metabolites such as amino acids, enzymes/proteins, polysaccharides, vitamins and other organic compounds. These bio-molecules can act as reducing, capping and stabilizing agents either individually or collectively, to dictate the size and shape of particles [7,8].

Silver nanoparticles (AgNPs) are broadly used in medical fields to treat infections [9–14]. The dental caries are identified as tooth decay caused by acidogenic species of bacteria such as *Streptococcus mutans*, *Lactobacillus* and *Actinomyces*. The clinical signs are the loss of the mineralized tissues, which consequently lead to pain, damage to the structure of teeth, and eventually the loss of the entire tooth, both in children and adults [15,16]. The periodontal diseases are primarily associated with *Actinomyces*, *Lactobacillus*, *Streptococcus* and *Candida* species, which are involved in the inflammation and infection and destroy the tissues that support the teeth, including gums, periodontal ligaments, and the alveolar bones [17,18]. In recent years, many works

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have been carried out for the fabrication of new antimicrobials from various bio-sources such as microorganisms, animals, and medicinal plants [19,20].

Justicia is a genus of flowering plants in the family Acanthaceae, which is found in pan tropical and tropical regions and recognized as scandent perennial herbs or sub shrubs [21]. The perennial herb *Justicia glauca* is used in folk medicine to develop new therapeutic agents with cytotoxic activity [22]. It is used as a drug and an antidote for venomous stings and bites, diarrhea, dysentery, dropsy and swellings [23]. The phytochemical study of *J. glauca* indicates that it contains lignans such as (+)-pinoresinol, (+)-medioresinol, (+)-lariciresinol, (+)-isolariciresinol, (+)-8-methoxyisolariciresinol, justiciresinol, and sitosterol-3-0-glucoside. Lignans are larger groups within *J. glauca* that show diverse biological activities such as antiangiogenic, antileishmanial, antifungal, hypolipidemic, antiasthmatic, antiviral, antineoplastic, antifeedant, insecticidal, cardiotonic, antidepressant, analgesic, antiplatelet and anti-inflammatory [24,25]. The lignan lariciresinol is a major constituent in *J. glauca*, which is a cytotoxic substance as well as insect antifeedant [26]. In addition, the Lignan justiciresinol demonstrates low cytotoxicity against three human tumor cells, such as lung carcinoma, breast carcinoma and colon adenocarcinoma [27].

In recent times, the researchers have brought to light the new approach to study the combined effects of antibiotics and green synthesized antimicrobial nanoparticles [28–30]. These new approaches facilitate to improve antimicrobial activity and potentially overcome resistance to the current antibiotics [31]. The present study demonstrates a green synthesis of AgNPs extracellularly using *J. glauca* leaf extract. The antimicrobial activities of green synthesized AgNPs and drug blended AgNPs have been evaluated against the dental caries and periodontal disease causing microorganisms such as *S. mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

2. Experimental

2.1. Materials and methods

All the media components and analytical reagents were purchased from Hi-Media Laboratories Pvt Ltd. (Mumbai, India) and Sigma Aldrich Chemicals (St. Louis, USA). The leaves of *J. glauca* were collected from Sirumalai hills region, Tamil Nadu, India. The dental caries/periodontal disease causing microorganisms such as *S. mutans*, *S. aureus*, *L. acidophilus*, *M. luteus*, and *B. subtilis*, gram negative bacteria such as *E. coli*, *P. aeruginosa* and fungal culture, such as *C. albicans* were obtained from Microbiology laboratory, SRM University hospital and research center, Chennai, India.

2.2. Preparation of J. glauca leaf extract

The leaves of *J. glauca* were washed to remove adhering mud particles and possible impurities. Afterward the leaves were spread over filter paper to remove the wetness of leaf and then dried at room temperature for an hour. 0.3 g of leaves was weighed and sliced in tiny size. These tiny pieces were boiled in 300 mL of sterile distilled water in an Erlenmeyer flask for 15 min and allowed to cool at room temperature. The boiled leaf extract was then filtered twice using

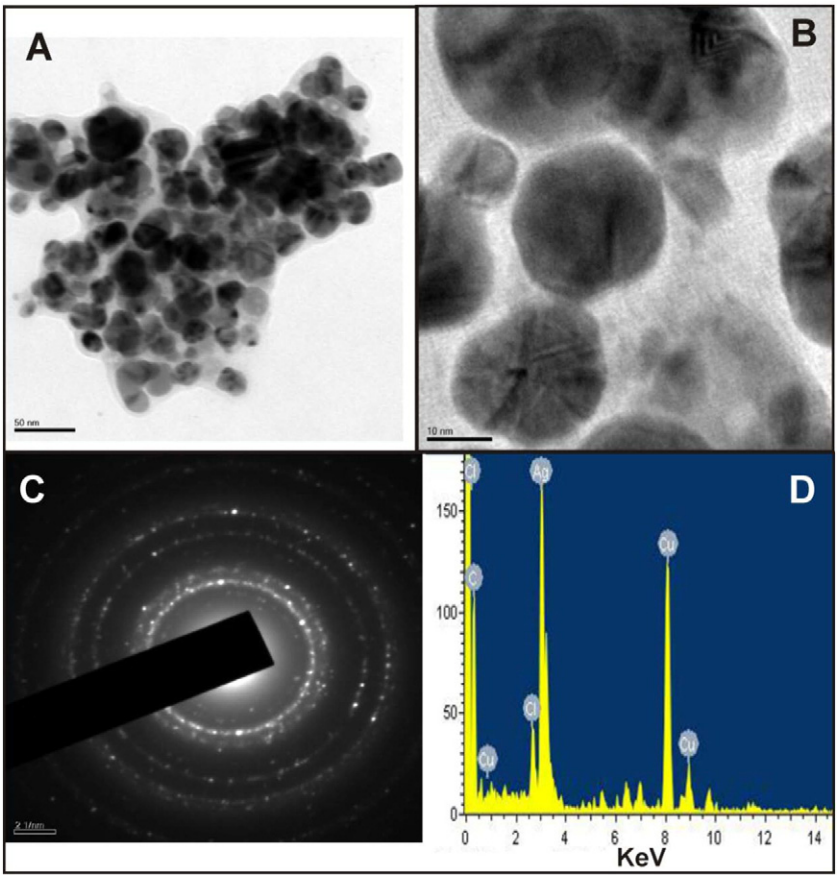

Fig. 1. The TEM image of green synthesized AgNPs (A) in 50 nm scale and (B) in 10 nm scale. (C) The corresponding Selected Area Electron Diffraction (SAED) patterns and (D) EDX profile of AgNPs.