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Antibacterial efficacy of silver nanoparticles against multi-drug resistant clinical isolates from post-surgical wound infections

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ABSTRACT

In order to investigate new effective and inexpensive nano-therapeutic approach for *P. aeruginosa, staphylococcus aureus* and coagulase negative staphylococci (CoNS), the present study reports an eco-friendly process for rapid synthesis of silver nanoparticles (Ag-NPs) using aqueous leaf extract of *Corchorus Capsularis* (CRCP). Formation of stable Ag-NPs at different time intervals gives mostly spherical particles with diameters ranging from 5 to 45 nm. The resulting Ag-NPs were characterized using Ultraviolet visible (UV–Vis) spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, X-Ray Diffraction (XRD) analysis, Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray analysis (EDX). XRD study shows that the particles are crystalline in nature with face centered cubic geometry. TEM studies show the formation of Ag-NPs with average size of 20.52 nm. The antimicrobial activity of the synthesized Ag-NPs was investigated against multi drug resistant (MDR) *P. aeruginosa*, *Staphylococcus aureus* and CoNS isolates from post-surgical wound infections. The present study suggests that Ag-NPs synthesized from aqueous leaf extract of CRCP shows significant antibacterial potential against MDR isolates from post-surgical wound infections.

1. Introduction

Post-surgical wound infections are global problem in the field of surgery associated with long hospital stay, higher treatment expenditure, morbidity and mortality [1–3]. Most post-surgical wound infections are hospital-acquired and vary from one hospital to the other [4]. Studies have reported that the prevalence of post surgical wound infections range from 14.8% to 60% which are caused by *S. aureus*, CoNS and *P. Aeruginosa,* the most common pathogens [3,5]. Lack of standardised criteria for diagnosis presents a challenge to monitor the global epidemiology of surgical site infection. In addition to this, emerging of high anti-microbial resistance among bacterial pathogens has made the management for the treatment of post-operative wound infections difficult [6]. Various biological methods have been proposed by exploiting microorganisms [7] and vascular plants [8,9]. The Ag-NPs are reported to be nontoxic and most effective materials against bacteria, viruses, and other eukaryotic micro-organisms at very low concentrations without any side effects [10]. Several physical and chemical processes [11–16] for synthesis of metal nanoparticles (MNPs) have been developed considering the real life application in the various biomedical areas [17], catalysis [18], detection [19], storage batteries [20] etc. The studies of MNPs are moving toward the exploration of benign green methods for synthesis and application in antibacterial, antioxidant and antitumor activity. Biosynthetic processes have received much attention as a viable alternative for the development of MNPs, where plant extract is used for the synthesis without any chemical ingredients [21–25]. It is well known that nano-silver possesses a strong microbicidal activity against bacteria, fungi and viruses [26]. The high antibacterial activity of Ag-NPs is a result of well-developed surface and providing maximum contact with the microbial environment [27]. In the present work, we report the synthesis of Ag-NPs using leaf extract of CRCP as both reducing and stabilizing agent. The leaves of CRCP have been widely used as stimulant, laxative, appetizer, demulcent and stomachic and its mixture is commercially used to treat fever, constipation, dysentery, liver disorders and dyspepsia.

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Furthermore, a decoction of the root or unripe fruits has also been used to cure dysentery [28]. Biologically developed Ag-NPs have been characterized by XRD, TEM, EDX, SAED and UV–vis spectroscopy. The interaction of the biomolecules present in the extract and Ag-NPs were confirmed by FT-IR spectroscopy. A sufficient study of antimicrobial activity has been made on the basis of disk diffusion and growth kinetics using different concentration of Ag-NPs. Determination of growth kinetics assay, cytotoxicity assay and bactericidal activity assay has also been made based on the liquid culture of *P. aeruginosa*, *Staphylococcus aureus* and CoNS isolates from post-surgical wound infections.

2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$) (>99.9% pure), was obtained from Merck (India). All reagents used in this research were analytical grade and used without further purification. Throughout the procedures, double deionized water with a measured resistivity of 18.2 $MUcm^{-1}$ was used. The medicinal plant leaf of CRCP used for the synthesis of Ag-NPs was collected in the Thiagarajar College campus, Madurai, Tamilnadu, India. The antibacterial experiments were carried out using bacterial strains resistant *P. aeruginosa, Staphylococcus aureus and* Coagulase negative Staphylococci (CoNS) isolates from post-surgical wound infections.

2.2. Green synthesis of Ag-NPs

The leaf of CRCP was cleaned to remove the adhering mud particles and other possible impurities. Subsequently the leaves were laid on the filter paper to eradicate content of any moisture in the leaves and air-dried at room temperature for an hour. 6 g leaves were weighed and cut into tiny pieces. Afterwards the leaves were boiled at 90 °C with 300 mL of sterile distilled water in an Erlenmeyer flask for 15 min and allowed to cool at room temperature. The boiled and cooled leaf extract was double filtered. The leaf extract in pale yellowish color was used for green synthesis of Ag-NPs. For the synthesis of Ag-NPs, 150 mL of an aqueous solution of 1 mM $AgNO_3$ was added to the Erlenmeyer flask containing 150 mL CRCP leaf extract. After 10 min the leaf extract turns into a brown color indicating the formation of Ag-NPs. The brownish color becomes denser and after an hour, there is no noticeable change in the color. The color changes indicate the formation of Ag-NPs in an aqueous solution due to excitation of the surface Plasmon vibration in the MNPs. The synthesized Ag-NPs were collected by centrifugation at 2000 rpm for 10 min and then the filtrate was redispersed in water and centrifuged several more times to remove any excess amount of organic contents. Later the Ag-NPs were centrifuged and washed thrice with deionized water and lyophilized. After lyophilisation, the Ag-NPs were stored in a screw cap bottle for further characterization and application.

2.3. Characterization of synthesized Ag-NPs

The size and morphology of the Ag-NPs were examined by transmission electron microscopy (TEM, JEOL JEM 2100) attached with EDX spectrometer was used for elemental analysis. FT-IR was carried out using the Shimadzu FT-IR-8201 PC instrument. A Jasco V-560 double-beam spectrophotometer was used for UV–visible spectral analysis. X-ray diffraction (XRD, PW3050/60) was performed on the purified Ag-NPs with data analysis by XPERT-PRO.

2.4. Collection of clinical isolates and growth conditions

Swab samples were collected from the suspected person showing clinical signs of post-surgical wound infection in Ayder Referral and Teaching Hospital, Ethiopia. Infected site was cleaned using normal saline and sterile gauze. Two wound swabs were collected using sterile cotton swabs from the patient and then immediately transported and processed in the bacteriology laboratory in the department of Medical Microbiology, College of Health Sciences, Mekelle University, Ethiopia within 1 h of collection. The first wound swab was used to make Gram stain smears whereas the second one was inoculated into Blood agar, Mac Conkey agar and Mannitol-salt agar and incubated at 37 °C for 24–48 h. Identification of Gram positive bacteria was done using Gram stain, catalysis reaction, coagulase test and hemolytic activity on sheep blood agar plates. Gram-negative bacteria were identified based on colony morphology on Blood agar, Mac Conkey agar and then followed by biochemical reactions namely oxidase, triple sugar iron (TSI), Sulphur Indole, motility (SIM), citrate, urease tests [29]. The predominant bacterial isolates were identified as *P. aeruginosa*, *Staphylococcus aureus* and CoNS. These bacterial strains were sub cultured time to time in order to regulate the viability and were maintained on Muller Hinton agar slants in the microbiology laboratory which was stored at 4 °C for antimicrobial efficacy study using Ag-NPs. This study was approved by Ethical Review Committee of Mekelle University, College of Health Sciences (# REC REF 2012-127). Bacterial strains were further sub-cultured on Mueller-Hinton broth (pH 7.3 ± 0.2; Oxiod, Hampshire, UK) at 37° C with shaking at 150 rpm. Cell suspensions were diluted with a sterile saline solution to obtain a final concentration of 10^7 CFU/mL by comparison with a 0.5 McFarland turbidity standard for further antimicrobial efficacy study using Ag-NPs.

2.5. Disc diffusion assay

Disc diffusion assay was performed to check the antibacterial efficacy of Ag-NPs against CoNS and MRSA isolates from wound infected patients. To determine the antibacterial activity of Ag-NPs, inoculum of each bacterial isolates were prepared by aseptically and cell density was adjusted to 0.5 McFarland turbidity standards and then the inoculum was transferred and spread evenly on a Mueller Hinton agar (MHA) plates. Whatman No-1 filter paperdisc at 6 mm dimension was impregnated with Ag-NPs of various concentrations (20, 40 and 60 μg/mL) and allowed to dry 30 min in room temperature aseptically. The disc impregnated Ag-NPs were placed on the inoculated MHA plates and incubated in an inverted position for 24 h at 37° C. Standard antibiotic disc Gentamycin (15 μg) was used as a positive control (Oxiod, Hampshire, UK).

2.6. Bactericidal activity of Ag-NPs against P. aeruginosa, Staphylococcus aureus and CoNS

To determine the bactericidal activity of Ag-NPs, 10^6 CFU of *P. aeruginosa, Staphylococcus aureus* and CoNS isolates of post-surgical wound infection were treated individually with different concentrations of Ag-NPs (20, 40, 60, 80, 100 μg/mL) and control of 100 μg/mL of lyophilized plant extract, which is free of Ag-NPs. These preparations were incubated at 37 °C for 1 h and plated on LB agar plates. The plates were incubated at 37 °C for 24 h and the colonies were counted in an automated colony counter.

2.7. Determination of growth kinetics of P. aeruginosa, Staphylococcus aureus and CoNS

The bacterial growth kinetics was monitored to check the effect