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Antimicrobial activity of latex silver nanoparticles using *Calotropis procera*

Nadia Hussein Mohamed[1,2], Mady Ahmed Ismail[1], Wael Moustfa Abdel–Mageed[3,4], Ahmed Abdelfattah Mohamed Shoreit[1*]

[1]*Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt*

[2]*Biology Department, Faculty of Science and Art, Samtah, Jazan University, Saudi Arabia*

[3]*Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh 11451, P. O. Box 2457, Saudi Arabia*

[4]*Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt*

PEER REVIEW

Peer reviewer
Dr. Chiara Pelillo, PostDoc at Callerio Foundation Onlus, Trieste, Italy.
Tel: 00390405588635
E–mail: chiara.pelillo@libero.it

Comments
The research presented is very interesting and quite complete. The physico–chemical parameters of these latex silver NPs were well characterized. The antimicrobial activity was evaluated following the standard test used and against many different bacterial and fungi strains.
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ABSTRACT

Objective: To synthesize silver nanoparticles (AgNPs) by green methods using serum latex of *Calotropis procera* at 80 °C and evaluate them against bacteria, dermatophytes and phytopathogenic fungi comparing with the activity of untreated latex.
Methods: The synthesis of AgNPs was performed by mixing 3% latex serum extract with the same volume of silver nitrate (2 mmol/L) solution in round flask and heating in water bath at 80 °C. Characterization of silver particles were determined using UV–vis spectrophotometer, transmission electron microscopy (TEM), X–ray diffraction and Fourier transform infrared spectroscopy. The antimicrobial activity of the green synthesized AgNPs was determined against bacteria, dermatophytes and phytopathogenic fungi and compared to the crude untreated latex by agar–well diffusion methods.
Results: Biosynthesis of latex silver nanoparticles was successfully obtained by green method. The formation of AgNPs has been confirmed by UV–vis, TEM microscopy, X–ray diffraction and Fourier transform infrared spectroscopy. TEM analysis showed that synthesized AgNPs are highly stable spherical shaped particles, well dispersed with a diameter ranged from 4 nm up to 25 nm and an average size of 12.33 nm. AgNPs showed strong antibacterial activity against Gram–negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia* sp.) and antifungal activity against *Trichophyton rubrum*, *Candida albicans* and *Aspergillus terreus*.
Conclusions: It can be concluded that serum latex of *Calotropis procera* was found to display strong potential for the synthesis of AgNPs as antimicrobial agents through rapid reduction of silver ions (Ag^+ to Ag^0). The green synthesized AgNPs were found to show higher antimicrobial efficacy than crude latex.

KEYWORDS
Green synthesis, *Calotropis procera*, Latex, Silver nanoparticles, Antimicrobial activity

1. Introduction

Nanotechnology is an important tool in many fields, like health and medicine[1]. Nanotechnology is the technology of materials having particle size below hundred nanometers. The properties of materials below hundred nanometers usually differ from those in the bulk scales[1]. Silver nanoparticles (AgNPs) among all noble metals have been widely used in many pharmaceutical and biological applications because of its unique antimicrobial

*Corresponding author: Ahmed Abdelfattah Mohamed Shoreit, Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt.
Tel: +201003406161
Fax: +2088 2342708
E–mail: ashoreit1968@ yahoo.com
Foundation Project: Supported by Assiut Universityv, Assiut, Egypt..

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properties[1].

Biosynthetic green methods used for synthesis of nanoparticles usually involve using of medicinal plants and microorganisms such as fungi and algae to synthesis of nanoparticles for pharmaceutical and biological applications. It is eco-friendly, cost effective as compared to the other chemical and physical methods. It was also interesting to note that silver nanoparticles were able to exert inhibitory effect at a concentration that is below their cytotoxic limits. So they were regarded as safe to be used as antimicrobials[2-6]. The AgNPs prepared using green methods have high surface area, a smaller size and high dispersion and show a strong bactericidal and antibiotic activity. The AgNPs have several important applications in the field of antimicrobial agents, capable of purifying drinking water, degrading pesticides and killing human pathogenic bacteria[7-10]. Many recent reports were published on biosynthesis of AgNPs using plant latex[11-13], natural rubber latex[14,15], plant extract or by the whole plant showing promising biological activities[6,16], such as cytotoxic and antimicrobial activities.

Calotropis procera (family: Asclepiadaceae) (*C. procera*) is a cultivable wild xerophytic shrub found across Africa, Asia and South America[17]. It produces milky white latex that exhibits diverse curative properties[18-20]. Latex is found in special branching tubes called latex tubes[21,22], and has been the subject of interest due to its biological activities such as antibacterial[23], antifungal[24], antiviral[25], anticandidal[26] and anticarcinogenic activities[27,28]. More than 80% of the dry mass of the crude latex corresponds to rubber and the rest 20% covers soluble fractions rich in protein including antioxidant enzymes, cysteine protease with free thiol group and tryptophan[29,30].

In the present study, green nontoxic eco-friendly rapid method for synthesis of AgNPs using serum latex of *C. procera* plant at 80 °C was characterized and evaluated against bacteria, dermatophytes and phytopathogenic fungi and compared to the activity of untreated latex.

2. Materials and methods

2.1. Sampling of crude latex

Latex from *C. procera* grown in Assiut region, Egypt was drawn by sterile disposable syringe under sterile conditions into sterile Eppendorf tubes in July 2012.

2.2. Fractionation of plant latex

Fresh latex was centrifuged at 17 000 r/min for 20 min at 4 °C in SR4000 Prolabo centrifuge (made in France). It was separated into three layers: rubber, serum and lipids.

2.3. Synthesis of latex silver nanoparticles (LAg-NPs)

Separately, 25 mL of 3% latex serum extract diluted with deionized water was mixed with the same volume of silver nitrate (2 mmol/L) solution in round flask, heated in water bath at 60 °C with constant stirring for 15 min. The temperature was raised to 80 °C and the mixture was further incubated in water bath for 30 to 45 min until LAg-NPs was formed and the brownish yellow color of solution became stable.

2.4. Characterization of LAg-NPs

2.4.1. UV-vis spectral analysis

The reduction of pure Ag^+ ions by latex was monitored by periodic sampling of the reaction medium and measuring the UV-vis spectra of the solution at frequent time intervals using UV-vis spectrophotometer (Shimadzu model UV-1601) in range of 200 nm to 800 nm.

2.4.2. Transmission electron microscopy (TEM) analysis

The morphology of the AgNPs were investigated by TEM using JEOL-JEM-100 CXII instrument by drying a drop of the washed colloidal dispersion onto a copper grid covered with a conductive polymer.

2.4.3. Laser diffraction particle size analyzer

Particle size of AgNPs was analyzed on particle size analyzer system [Horiba LA-300 Light Scattering Particle Size Distribution Analyzer (Horiba Ltd, Kyoto, Japan)]. The average distribution of nanoparticles on the basis of intensity, volume and number weighting was studied comparatively.

2.4.4. X-ray diffraction (XRD) analysis

XRD was performed using X-ray diffractometer (Model PW 1710 control unit Philips Anode material Cu, 40 KV, 30 M.A, optics: Automatic divergence slit) with Cu Kα radiation λ=1.540 5 °A over a wide range of Bragg angles (30°≤2θ≤80°). An elemental analysis of the sample was examined by energy dispersive analyses of X-rays with JED-2300 instrument.