ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES BY SIMAROUBAGLAUCA AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Objective: The present study outline the plant-mediated synthesis of silver nanoparticles (AgNPs) using leaf extract Simaroubaglauca, which act as both reducing and stabilizing agent.

Methods: Formation of silver nanoparticles was confirmed by primarily by Ultraviolet/visible spectroscopy. X-ray diffraction studies revealed the crystallinity of the nanoparticles. The scanning electron microscopy was carried out to determine the mean particle size, as well as the morphology of the NPs and the composition of elements, was studied with Energy Dispersive X-ray analysis (EDS).

Results: The silver nanoparticles were spherical in shape with a mean size of 23 nm. The EDS showed strong optical absorption peak at 3keV and it was confirmed the formation of AgNPs. The synthesised AgNPs further utilized for the evaluation of antibacterial activity and shown significant antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Enterobacter and Klebsiella pneumonia at 50 µg/ml and 100µg/ml concentrations.

Conclusion: The synthesised silver nanoparticles have been characterised by UV-vis, SEM-EDAX and XRD to determine the sizes and shapes of the silver nanoparticles.

Keywords: Simaroubaglauca, Silver nanoparticles, Leaf extract, Bioreductant, Antibacterial

INTRODUCTION

Every person will suffer from either one or other diseases caused by various bacteria at least once in his or her lifetime that shows resistance against one or two existed antibiotics and which leads a severe public health problem [1, 2].

Hence, there is top-priority to develop alternative treatments for bacterial diseases. Silver was being used in the field of medicine for antimicrobial applications such as burn therapy [3, 4], removal of microbes on textile fabrics [5-7], and inhibition of colonization of bacteria on catheters [8-10]. As antimicrobial agents, Nano-silver systems offer many advantages. They own a very high effect towards a broad range of microbes and parasites, even at low doses and does not shows toxicity in humans and relatively inexpensive.

Thus Silver has been suspended within a wide variety of materials, under various forms such as salts, immobilized ions or metallic nanoparticles [11-13]. Plasma membrane, many important enzymes and DNA of the bacteria are important targets for silver ions [14-18].

Simaroubaglauca is one of the important traditional medicinal plants due to the presence alkaloids, flavonoids, carbohydrates, glycosides, a phenolic compound, tannins, terpenoids, cardenolides, saponins, fixed oils which can usually account for their therapeutic action including Antimicrobial, antiviral, anti-inflammatory, antiparasitic and antitumor activities [19]. But never synthesized and characterized silver nanoparticles by the extracts of Simaroubaglauca.

Here, we synthesized silver nanoparticles using an aqueous leaf extract obtained from Simaroubaglauca. In addition, to consider the biological application of our work, antibacterial evaluation was carried out.

MATERIALS AND METHODS

Preparation of leaf extract

Silver nitrate (AgNO3) was purchased from sigma-aldrich and fresh Simaroubaglauca leaves were collected from Shridevi Institute of Engineering and Technology Campus in Tumakuru of Karnataka, India. The collected leaves were washed thoroughly and cut into small pieces. Finely incised SIMAROUBAGLAUCA leaves (20g) were weighed and transferred to 500 ml conical flask containing 100 ml of distilled water and gently mixed and boiled for 5 min.

The obtained extract was collected filtered through Whatman No.1 filter paper and the filtrate was collected in 250 ml Erlenmeyer flask and stored at 4 °C for further use.

Fig. 1: Simaroubaglauca
Synthesis of silver nanoparticles using leaf extract

Silver nanoparticles were synthesized by mixing 5 ml of Simaroubaglauca leaf extract with 50 ml of silver nitrate aqueous solution (1 mmol) and stirred for 10 min at 30 °C. Reduction rapidly occurs as indicated by a pale bluish colour after 30 min indicating the formation of silver nanoparticles. The obtained silver nanoparticles were purified by centrifuging in a Remi cooling centrifuge at 10,000 rpm for 30 min. The pellets obtained were dispersed in deionized three times to remove water solubles.

Characterization of synthesized silver nanoparticles

UV-Vis spectral analysis

Biological mediated reductions of silver salts were monitored by UV-Vis spectroscopy. Absorption spectra were obtained using Shimadzu company model-UV3600 UV-Vis spectrophotometer using quartz cuvette and distilled water as a reference. Spectral readings of UV-Vis were recorded between 350 and 550 nm for synthesized silver nanoparticles.

Scanning electron microscopy (SEM) and EDAX

Size, shape and morphology of silver nanoparticles were resolved by scanning electron microscopy by using ZEISS Ultra 55 SEM machine operated 5 kV at Center for Nanoscience and Engineering, Indian Institute of Science, Bengaluru. A thin film of the sample was prepared on a carbon coated copper grid by just keeping the very small amount of the sample on the grid. The readings and photographic scan were taken at 50.00, 75.00 and 100.00 magnification with constant voltage and at different angles.

X-ray diffraction measurements (XRD)

Crystalline metallic properties of AgNPs were examined by XRD (Rigaku, SmartLab X-ray Diffractometer) at Center for Nanoscience and Engineering, Indian Institute of Science, Bangalore at a voltage of 40 keV and a current of 30 mA with Cu Kα radiation, step size=0.02, speed=5°/min with a wavelength of 1.5418 Å and at 2θ angle.

Antimicrobial activity of silver nanoparticles

Microbial cultures: Escherichia coli, Pseudomonas aeruginosa, Enterobacter and Klebsiella pneumonia were collected from Department of Microbiology of Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka of India and were maintained in nutrient broth.

The antimicrobial assay was carried out by Agar well diffusion method. Overnight samples were swabbed on the plates containing Muller Hinton Agar (MHA) medium (Hi-Media). Wells were prepared in the medium using sterile gel puncture.50 µg/ml and 100 µg/ml of synthesized silver nanoparticle solution was added to wells. Sterile distilled water and antibiotic ampicillin were added as negative and positive control respectively. Petri plates were kept for incubation for 24 h at 37 °C.

Statistical analysis

All the experiments were performed in triplicate, and the data were expressed as mean.

RESULTS AND DISCUSSION

The extracellular green synthesis of silver nanoparticles occurred during the exposure of the leaf extract to 1 mmol aqueous silver nitrate solution. The complete reduction of silver ions was observed 2-3h. The color change of the reaction was observed during the incubation period because the formation of silver nanoparticles is able to produce a specific color of the reaction mixtures because of their unique properties. The appearance of bluish color is a conclusive indication of the formation of silver nanoparticles in the reaction mixture (fig. 2). Due to the presence of coherent excitation of all the ‘free’ electrons within the conduction band, the color could be exhibited by the metal particles and leading to an in-phase oscillation which is known as surface plasmon resonance (SPR) [20].

![Fig. 2: Simaroubaglauca leaf extract, before and after treating with silver nitrate](image)

UV-vis spectroscopy technique was applied to monitor the SPR absorbance band of silver nanoparticles synthesized using leaf extract of Simaroubaglouca and it was centered at 440 nm and relentlessly increments in intensity as a function of the time of reaction without any shift in the peak wavelength (fig. 3) [21]. The frequency and width of the surface plasmon absorption relies on the size and shape of the metal nanoparticles and in addition on the dielectric constant of the metal itself and the encompassing medium.

![Fig. 3: UV-vis spectrum of silver nanoparticles synthesized using leaf extract Simaroubaglauca](image)

**Fig. 4: SEM photographs of silver nanoparticles obtained using leaf extract Simaroubaglauca, a. 50.00, b. 75.00, c. 100.00**
The microstructural qualities of the synthesized silver nanoparticles were studied by SEM (fig. 4). Little round shaped outgrowths of silver nanoparticles agglomerates with homogenous dissemination. The silver nanoparticles were generally circular, and the size was evaluated to be in the range in the vicinity of 23 and 49 nm. The EDX investigation of silver nanoparticles affirms that the nanoparticles are in fact silver nanoparticles (fig. 5). It demonstrates that silver nanoparticles were upheld on the natural grid from the *Simaroubaglauca*. The peak of Ag component is most likely from the silver nitrate.

Antibacterial activities of Au and Ag nanoparticles

In our investigation, the synthesized silver nanoparticles have been evaluated for their antibacterial activities against human pathogens such as Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* and *Klebsiella pneumonia* at (50 µg/ml and 100 µg/ml) different concentration. Silver nanoparticles shown zones of inhibition 30 mm and 32 mm, 5 mm and 8 mm, 4 mm and 6 mm and 3 mm and 5 mm against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* and *Klebsiella pneumonia* respectively at the concentration of 50 µg/ml and 100 µg/ml (fig. 7). Both the concentration of silver nanoparticles have indicated practically comparative outcomes have appeared by standard amphicillin against *Escherichia coli* whereas other pathogens against which have failed to show significant results. Silver nanoparticles have shown their effect against *Escherichia coli* a Gram-negative bacteria and it was suggested that the effect would be size and dose dependent [24].
CONCLUSION
The medicinally significant aqueous leaf extract of *Simaroubaglauca* was found to behave as a reducing agent for the production of silver nanoparticles. The synthesized silver nanoparticles have been characterized by UV-vis, SEM-EDAX and XRD to determine the sizes and shapes of the silver nanoparticles. The developed method is one of the excellent methods to produce silver nanoparticles in absence of toxic reducing chemicals. Our outcomes suggest that the biologically synthesized silver nanoparticles are most prominent against human pathogens.

CONFLICT OF INTERESTS
Declare none

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