Volume 9, Issue 2, 2020, 1037 - 1041 ISSN 2284-6808

Letters in Applied NanoBioScience

https://nanobioletters.com/

https://doi.org/10.33263/LIANBS92.10371041

Original Research Article Open Access Journal

Received: 10.04.2020 / Revised: 04.05.2020 / Accepted: 08.05.2020 / Published on-line: 10.05.2020

Green synthesis and antibacterial activity of silver nanoparticles from the aqueous extracts of *Cassia alata*

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ABSTRACT

The current study involves the synthesis of silver nanoparticles from the aqueous extracts of *Cassia alata*. The synthesized silver nanoparticles were characterized by UV-Vis, FT-IR, SEM-EDX and XRD. The *in vitro* bioactivity studies were examined against a Gram-positive and Gram-negative micro-organism by performing antibacterial activity, minimum inhibition assay, swarming motility and protein leakage assay. The characteristic study on the nanoparticles using spectrometric and microscopic analysis, revealed them to be sized between 25 nm and 60 nm. The nanoparticles on evaluating the antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* interacted well with the microorganisms producing a significant inhibitory effect at a minimum concentration of 0.5 µg/mL. The permeability of the particles was also studied through protein leakage assay.

Keywords: *Silver nanoparticles; Antibacterial activity; MIC; Protein leakage assay; Cassia alata.*

1. INTRODUCTION

Plants are the richest asset of phytoconstituents that revolutionized the concept and scope of drug discovery. The future of pharmaceutical industry relies on the versatility and complexity of compounds derived from medicinal herbs [1-4]. Evidences support that plant crude extracts have been employed in the form of powder, solutions, etc to prevent and cure diseases since the conventional period. This has drawn the attention of researchers for extracting bioactive substances from plants and to use for drug discovery [5]. Formulation of herbal based nanomedicines is an added advantage including larger surface area, less toxicity, enhanced activity, stability, bioavailability, biocompatibility, sustained delivery etc [5-9]. Plant metabolites such as saponins, flavonoids, terpenoids, tannins produce an efficacious antimicrobial, antioxidant, anti-inflammatory results but are poorly absorbed through lipid layers because of high molecular size leading to less bioavailability [10,11]. To overcome these obstacles, herbal medicines are approached with nanotechnology for efficient drug delivery and action [12]. There are more active ayurvedic formulations, where metals formulations are used to treat diseases [4]. Synthesis of silver nanoparticles using biological means is being in practice for the past more than three decades [13]. The plant molecules can reduce the size, stabilize nanoparticle and in a mixture can produce a better result [13, 14]. Plant derived silver nanoparticles are reported to have various bioactivity [8,9]. The possibility of *Cassia alata* to reduce the metallic halide was checked by treating the aqueous extracts of the leaf to Silver nitrate. The procured nanoparticles were checked for its ability to interact with microbial cells and were checked in two species *Pseudomonas aeruginosa* and *Bacillus subtilis*.

2. MATERIALS AND METHODS

2.1. Processing of leaf sample.

The leaves collected were scrutinized macroscopically and authenticated by the Director of Plant Anatomy Research Centre, Dr. P Jayaraman from Chennai. The leaves were washed, shade dried and finely powdered.

2.2. Preparation of plant extracts.

The aqueous extraction was done by decoction method where 20g of the powdered leaf was boiled in deionised water for 10 min with constant stirring. The extract is then cooled and the menstrum was collected to be preserved at 4°C [15].

2.3. Synthesis of Silver nanoparticle.

The nano synthesis was carried out by mixing 5 mL of aqueous extract to 25 mL of silver nitrate. The bio reduction was maintained in dark at room temperature. A notable change in colour from pale yellow to dark brown would be observed after a period of 24 h, indicative of the nano synthesis [16, 17]. Once the colour change was observed, the solution was centrifuged at 5000 g and the pellet was collected, lyophilized and stored at 4°C.

2.4. Characterization of silver nanoparticles.

The initial characterization was performed in UV-Visible spectroscopy (UV – 1800 Shimadzu, Japan) by exposing the particles in the electromagnetic spectrum between 200 nm and 800

nm. Further, the functional group in the nanoparticles checked in FTIR spectrum over the transmittance range 4000-400 cm^{-1} (Alpha Bruker optic Gmbh instrument). The spectra were plotted with wave number on X-axis and transmittance on Y-axis. Size of the sample was analysed by Scanning Electron Microscopy (Zeiss, GeminiSem). The elemental compound determination was confirmed with EDX spectrometry. The nature of the nanoparticle as crystalline or amorphous was analysed by studying them in X-ray diffraction (XRD) in the range 20°– 80°at 2θ angles [18].

2.5. Interaction of nanoparticles with microorganisms in static condition.

The impact of the nanoparticle to interact with the microorganisms was studied in static conditions in an agar plate. The study was performed by cutting wells in the agar plate and loaded with *Pseudomonas aeruginosa* and *Bacillus subtilis*. The plates were maintained at 28^0 C for the growth of the microorganisms [19].

2.6. Interaction of nanoparticles with microbial suspension culture.

The silver nanoparticles were allowed to interact with the microorganisms, *Pseudomonas aeruginosa* and *Bacillus subtilis* which were maintained as a suspension culture. The study was carried out for different concentration ranging from 0.25 to 2 µg/mL and the least concentration of the silver nanoparticles that inhibits the growth was denoted as MIC (minimum inhibitory concentration) [20].

2.7. Swarming motility.

Swarming motility of both *Pseudomonas aeruginosa*, *Bacillus subtilis* in the presence of silver nanoparticles (0.5µg/ mL) was performed following the method of Ugurlu *et al* [21] and Samrot et al [22].

2.8. Protein leakage assay.

The ability of the nanoparticle to permeate the membrane of live cells is studied by allowing them to interact which is evidenced by protein leakage from the microbes. This study was conducted on two species *Bacillus subtilis* and *Pseudomonas aeruginosa* following the procedures of Gunalana *et al* [23] and Maruthai *et al* [24].

3. RESULTS

3.1. Characterization of silver nanoparticles.

The formation of silver nanoparticles was identified when the pale solution changed its colour to dark brown after the incubation with silver nitrate. The spectral peaks analysed by the FT-IR analysis resulted in a broad trough at 3270 cm^{-1} corresponded to OH-stretching (Fig. 1) [8, 25]. The intense band observed at 2920 cm^{-1} indicated the symmetric CH_2 stretching and the peak near 1600 cm^{-1} denoted the amide bonds which might be conferred by plant metabolites [25]. The spherical shaped silver nanoparticle produced by *Cassia alata* plant extracts was revealed by SEM analysis and the particles were of 31.55 nm in size (Fig. 2). An intense peak at 3 keV in the EDX analysis was seen for metal element silver (Fig. 3). The crystallinity of the nanoparticle with face-centered cubic structure was determined by XRD (Fig. 4), high-intensity silver nanoparticle peaks were observed with diffraction points of 38°, 44.5°, 64.81°, 77.43° corresponding to (111), (200), (220), (311) planes [26, 27, 28].

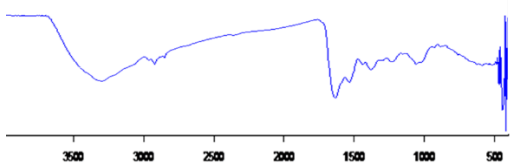

Figure 1. FT-IR analysis of Silver Nanoparticle synthesized by *Cassia alata.*

3.2. Interaction of nanoparticles with microorganisms in static condition.

The interaction of the nanoparticle with the bacterial cells studied on *Gram-positive (Bacillus subtilis)* and *Gram-negative (Pseudomonas aeruginosa)* organisms was found to have significant effect. It is observed that growth of the organisms was severely affected by the silver nanoparticles. (Table 1 and 2). Susceptibility was seen at 2 µg concentration itself. All the plant extract derived silver nanoparticles showed a potent antibacterial effect with the dose response in positive correlation.

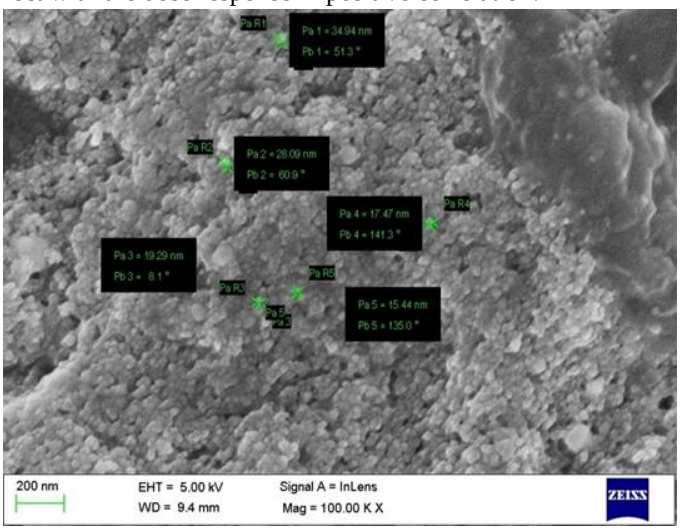

Figure 2. SEM analysis of Silver Nanoparticle synthesized by *Cassia alata.*

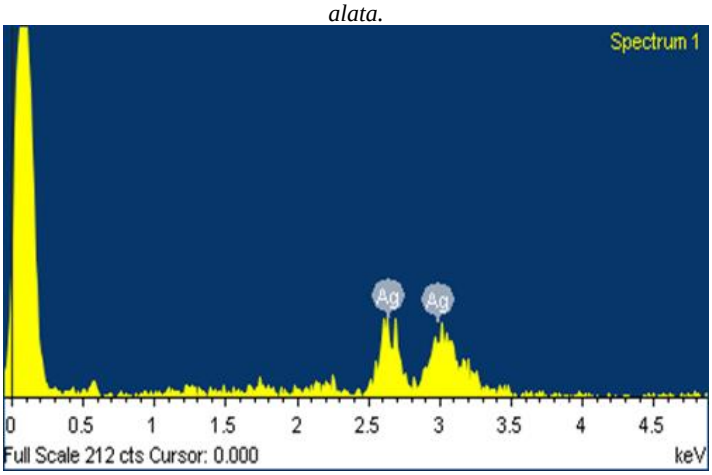

Figure 3. EDX of Silver Nanoparticle synthesized by *Cassia alata.*

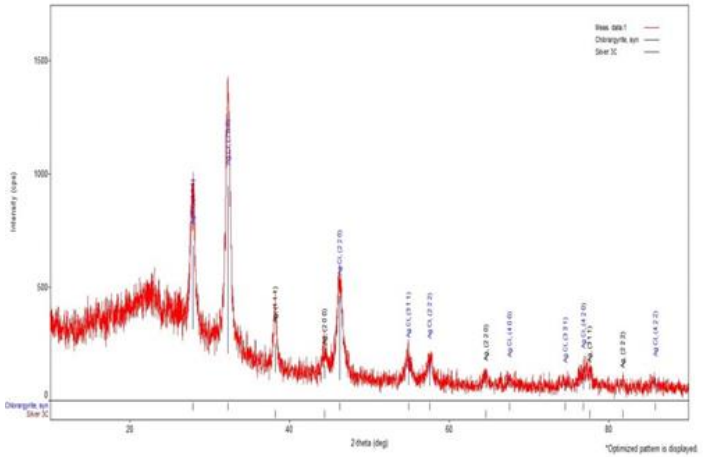

Figure 4. XRD analysis of Silver Nanoparticle synthesized by *Cassia alata.*

Table 1. Antibacterial activity of silver nanoparticles against *Pseudomonas aeruginosa.*

Components	Zone of inhibition (diameter in cm)
2 µg	0.8
4 µg	1.3
8 µg	1.0
16 µg	1.5
Positive control (Erythromycin)	1.1
Negative control (Water)	-ve

-ve indicates the lack of antibacterial activity

Table 2. Antibacterial activity of silver nanoparticles against *Bacillus subtilis.*

Components	Zone of inhibition (diameter in cm)
2 µg	0.4
4 µg	1.1
8 µg	1.6
16 µg	1.3
Positive control (Erythromycin)	3.1
Negative control (Water)	-ve

-ve indicates the lack of antibacterial activity

3.3. Minimum Inhibitory Concentration.

The minimum concentration of the plant derived nanoparticle that could suppress the growth of *Bacillus subtilis* (Table 3) when assessed for the four working concentrations revealed that 0.5 µg/mL of the nano concentration was effective on *P. aeruginosa*. Similar results were substantiated by reports on *Capsicum annuum* derived silver nanoparticles with analogous effect against *P. aeruginosa* [9]. Silver nanoparticles are reported to destabilize the membrane and leak the protein out of the cells [8, 23].

Table 3. Minimum Inhibitory Concentration (MIC) of crude silver nanoparticle.

Components	Zone of inhibition (diameter in cm)
2 µg	0.4
4 µg	1.1
8 µg	1.6
16 µg	1.3
Positive control (Erythromycin)	3.1
Negative control (Water)	-ve

-ve indicates the lack of antibacterial activity

3.4. Swarming motility.

The nanoparticle derived using *Cassia alata* was found to inhibit the motility of both *Bacillus subtilis* and *Pseudomonas aeruginosa* completely (Fig. 5 and 6). Nanoparticles could easily inhibited the motility of the organism.

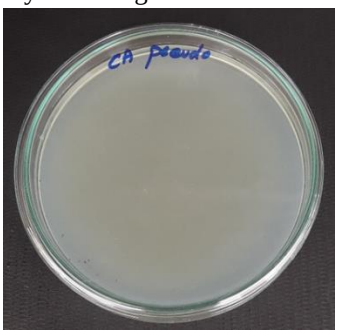

Figure 5. Swarming motility of *P. aeruginosa* against crude silver nanoparticle.

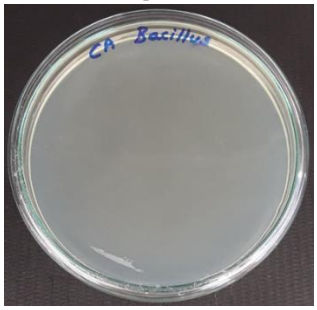

Figure 6. Swarming motility of *B. subtilis* against crude silver nanoparticle.

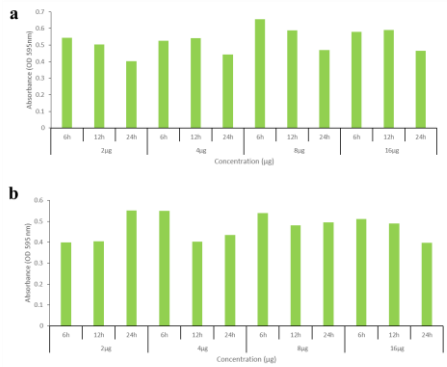

Figure 7. Protein leakage assay for silver nanoparticles against a) *P. aeruginosa* and b) *B. subtilis.*

3.5. Protein leakage assay of silver nanoparticles.

Increase in time and concentration increased the protein release profile in *P. aeruginosa* which was superior than the protein release report of *B. subtilis* (Fig. 7a, b), which correlated to similar

findings in other studies [8, 23]. The overall result suggested smaller sized silver nanoparticles could easily permeate *Gram-negative* cell wall than *Gram-positive* which made them sensitive to leak more amount of protein. Our results are on par with the results of Maruthai *et al* [21], who have also reported the cell wall disruption of silver nanoparticles reasoning their antibacterial effect. The silver nanoparticles synthesized using the crude extracts of *Thespesia populnea* and *Wrightia Tinctoria* also caused high protein release via cell disruption [29, 30].

4. CONCLUSIONS

In this study, aqueous extracts of *Cassia alata* were assessed for its capability to reduce metallic silver and also to test its ability to interact with live cells especially *B. subtilis* and *P. aeruginosa.* Since there is a pronounced impact of silver nanoparticles on both Gram positive and Gram negative bacteria, they can be used as a bactericidal agent.

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6. ACKNOWLEDGEMENTS

All the authors acknowledge The Management, Sathyabama Institute of Science and Technology for providing the lab.