Green Biosynthesis of AgNPs using Albizia saman Leaf Aqueous Extract and their Biological Applications

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ABSTRACT
Now days, nanotechnology has emerged as an simple division of current science and untied novel epoch in the fields of material science and receiving the worldwide attention due to its ample applications. The silver nanoparticles (AgNPs) have attracted with considerable interest as a result of their extensive applicability in different research fields such as chemistry, energy, medicine, and catalysis. In the present investigation, we have described a cost effective and eco-friendly technique for the synthesized AgNPs was completed using the aqueous extract of Albizia saman leaf and silver nitrate (1 mM) as a reducing property and also used as a capping agent. AgNPs were characterized using the spectral studies via, UV, FT-IR, XRD, EDX, and HR-SEM analysis. AgNPs were found the size ranging from 30 to 60 nm. AgNPs were also analyzed by ELISA testing method. A silver nanoparticle at different concentrations was evaluated for its antibacterial effect, against various pathogens. The MIC value (minimum inhibitory concentration) was increased with increasing concentration of AgNPs. Photocatalytic study of these synthesized AgNPs was evaluated using methylene blue as an organic dye, under sunlight irradiation and these nanoparticles showed the higher efficiency in degrading the dye within a few minutes of exposure. Further, biosynthesized nanoparticles also exhibited more significant cytotoxic effect on anticancer cell lines (MCF-7). Moreover, the biosynthesized AgNPs offer copious benefits of eco-friendly and compatibility for pharmaceutical, as well as studies on different biological activities in various fields should be strengthened in future.

1. Introduction
Nanotechnology approaches to prevent the diseases in humans and natural products have recently been increasing greatly, and exclusive the physicochemical activities of nanosized metal particles make it successful in natural science and medicine [1]. The various potential biological applications of nanoparticles have played a major role in the search for eco-friendly process of producing nanoparticles using various biomaterials, as the conventional
chemical synthesis involves the use of toxic solvents, energy, and high pressure which may be harmful to the environmental fields. The synthesis is efficient of yielding nanoparticles of sole features and property, which may influence their many applications.

Now, plants and plant-derived materials are used for nanoparticles synthesis which is more convenient than the microbe-mediated nanoparticles synthesis method because they eradicate the culture maintenance and are easy to handle [2]. Nanoparticles synthesis by medicinal plants indicates more advantage; they may enhance the antibacterial activity of silver nanoparticles because the medicinally important bioactive molecules present in the plants may bind on the surface of the nanoparticles and reduce the silver ions to AgNPs.

Due to their rich biodiversity of the plants, the significant biological material in the synthesis of nanoparticles is into the future completely explored. Synthesis of AgNPs is of much interest to the scientific population because of their wide range of the applications. These AgNPs are being fruitfully used in the cancer diagnosis and treatment as well [3,4]. Biological synthesis of AgNPs is a cost effective natural process of AgNPs synthesis, where the phytochemical especially phenols, flavonoids, and alkaloids present in the plant extracts acts as a capping and reducing agent. Due to their nanosize regime, the AgNPs may directly bind with DNA of the pathogenic bacterial strains leading to potent antimicrobial activity.

The aim of the present work is to synthesis of AgNPs using the aqueous leaf extract of *Albizia saman* (*A. saman*) as a reducing agent and stabilizer. AgNPs are characterized using UV–vis, FT-IR, XRD, EDX, and HR-SEM analysis. The influence of different process variables the silver nitrate concentrations, mixing ratio of the reactants is investigated on the biological synthesis of AgNPs by the aqueous leaf extract of *A. saman*. Hence, the antibacterial property of AgNPs is evaluated against three pathogenic bacteria strains, including *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), and *Escherichia coli* (Gram-negative) using the disk diffusion method. To the best of our knowledge, AgNPs is reported about the photo degradation of methylene blue dye by green synthesized AgNPs using *A. saman* leaf aqueous extract. Finally, these biologically synthesized AgNPs of *A. saman* leaf extract were found to produce a high cytotoxic activity.

## 2. Materials and Methods

### 2.1. Plant Authentification

Healthy and fresh leaves were collected from Dharmapuri district, Tamil Nadu. At the time of collection, a pressed sample was prepared and authenticated by a Botanist (BSI/SRC/5/23/2012-2013/Tech-83), Botanical Survey of India (BSI), Southern Circle, TNAU and Coimbatore.

### 2.2. Preparation of Plant Extract

*A. saman* leaves were chopped and shade dried for five days at room temperature. Dried leaves were washed with double-distilled water. 5 g of *A. saman* leaf powder was added to 100 mL of double-distilled water and heated for 1 h at 60 °C. The prepared aqueous extract was centrifuged and filtered through Whatman No. 1 filter paper. The filtered extract was stored in refrigerator at 4 °C.

### 2.3. Phytochemical Screening Analysis

*A. saman* leaf aqueous extract was subjected to qualitative preliminary phytochemical screening to evaluate the presence of the secondary metabolites according to the standard methods [5].

### 2.4. Biosynthesis of AgNPs

About 50 mL of AgNO₃ (1 mM) solution were added to 25 mL of aqueous leaf extract of *A. saman* and mixed on a magnetic stirrer. The reaction mixture was shaken to ensure thoroughly mixing and allowed to settle at room temperature. The light yellow color change to brown color confirmed the formation of AgNPs. The filtered solution was used immediately for AgNPs synthesis which served as reducing agent.

### 2.5. Characterizations

#### 2.5.1. UV–Vis Analysis

The change in color was visually obtained in AgNO₃ solution incubated with aqueous leaf extract of *A. saman*. The bio reduction of Ag⁺ ions was monitored by sampling of aliquots (2.5 mL) at various time variations. Absorption measurements were carried out on T80 UV–vis spectrometer at the ranges of wavelength in 200–800 nm.

#### 2.5.2. FT-IR Analysis

In the FT-IR spectrum, the samples were determined using the model of Perkin-Elmer in the transmittable mode at the ranges from 4000 to 400 cm⁻¹ in KBr pellets.

#### 2.5.3. XRD Analysis

XRD measurements, the synthesized AgNPs solution was drop-coated on to glass substrate and carried out on Model Rigaku miniflex II XRD operated at voltage for 40 kV and a current for 30 Ma with Cu-Kα (1.54 Å) at a room temperature.
2.5.4. **EDX Analysis**

The energy dispersive X-ray spectroscopy was performed by a Bruker EDX spectrometer to determine the elemental composition of the samples.

2.6. **HR-SEM Analysis**

The morphological of the synthesized sample, HR-SEM as a model in FEI Quanta FEG 200-HR-SEM of the biosynthesized AgNPs (boiled and centrifuged) samples were performed. The average particle size of the synthesized AgNPs was measured.

2.6.1. **In Vitro Release Study**

*A. saman* aqueous leaf extract was added to dispersion of AgNPs. The dispersion was incubated for 12 h at 2–8 °C followed by ultra centrifugation at 1000 rpm for 30 min. Thus obtained was separated from the supernatant solution and re-dispersed in milli-Q water prior to further analysis. The free leaf extract present in the supernatant was determined by ELISA testing method.

2.6.2. **Photocatalytic Property**

The catalytic activity of the biosynthesized AgNPs was prepared by hydrothermal method [6]. It was compared to degradation of Reactive Blue 160 (RB 160) in aqueous solution as a model system. The UV light was used as light source. In a typical photo degradation process, 20 mg sample was added to 10 ml of 1×10⁻⁴ M concentration of RB 160 solution. In various time intervals, at a wavelength of 382 nm the absorbance spectrum of the solution was measured using UV–vis spectrophotometer.

2.7. **Biological Applications**

2.7.1. **Antimicrobial Study**

The disk diffusion method [7] was used to display the antimicrobial properties. In *vivo* antimicrobial activity was tested using the agar plates obtained from Himedia. The agar plates were prepared by well 15 mL of molten media into sterile petriplates. The plates were qualified to solidify for 5 min and 0.1% inoculums suspension was swabbed evenly and the inoculums were allowed to dry for 5 min. The concentration of AgNPs 40 mg/disk was loaded on 6 mm sterile disk. The loaded agar disk was placed on the surface of medium and AgNPs was allowed to spread for 5 min and the plates were kept for incubation at 37 °C for 24 h. At the end of incubation, the zone of inhibition was formed to around the disk to measure with clear ruler in mL.

2.7.2. **Cytotoxic Activity**

Cytotoxic effect of the AgNPs was measured by the MTT assay [8]. The cells were maintained in DMEM medium, added with 10% of bovine serum at 37 °C in humidified atmosphere with 5% CO₂. The cells were plated in 96 well a flat bottom tissue culture plates at concentration of approximately 1.2×10⁴ cells/well and allowed to attach overnight at 37 °C. Then, the cells were incubated by different concentrations of the aqueous leaf extract of *A. saman* for 24 h. After the incubation, the medium was redundant and 100 μL fresh medium was added with 10 μl of MTT (5 mg/ml). After 4 h, the medium added in 100 μL of DMSO to dissolve the Formosan crystals. Then, the absorbance was read at 570 nm in a microlitre plate reader. The IC₅₀ value was calculated by the following formula.

\[
\text{Cell Inhibition (\%) = 100 - } \left( \frac{\text{ABS}_{(\text{AgNPs})}}{\text{ABS}_{(\text{Control})}} \right) \times 100
\]

2.7.3. **Statistical Analysis**

All the measurements were carried out in triplets and the results are expressed as mean ± SD using one way analysis (ANOVA) through SPSS software (16.0 version).

3. **Results and Discussion**

3.1. **Phytochemical Qualitative Screening Tests**

The results of preliminary qualitative phytochemical screening analysis are shown in Table 1. The preliminary phytochemical analysis may be valuable in the detection of the bioactive compounds and next may lead to the drug finding and development. Further, so it has been used for the biosynthesis of silver nanoparticles.

3.2. **Synthesis of Silver Nanoparticles**

Silver nanoparticles (AgNPs) were synthesized according to the established protocols using *A. saman* leaf, resulting in the type of nanoparticles AgNPs. In this present study, we attempted the fabrication of AgNPs using the precipitation method. The addition of leaf extract to the

| Table 1. Phytochemical qualitative screening analysis. |
|-----------------------------------------------|
| **Phytoconstituents** | **Albizia saman** | **Aqueous leaf extract** |
| --- | --- | --- |
| Alkaloids | + | |
| Flavonoids | + | |
| Phenolics | + | |
| Tannins | + | |
| Saponins | − | |
| Carbohydrate | + | |
| Proteins | + | |
| Steroids | + | |
| Terpenoids | + | |
| Glycosides | + | |
| Fats/Oils | − | |

Note: +; presence, −; absence.
3.3. Characterizations

3.3.1. UV–Vis Analysis
The aqueous leaf extract of *A. saman* with aqueous solution of the silver nitrate (AgNO₃), begin to change the color from brown to reddish-brown color. It indicated the formation of AgNPs with reduction of silver ion. The absorption bands were obtained at 262 nm (Figure 2). It shows the effect of optimization to 1 mM concentration of AgNO₃ solution. It was generally recognized that UV–vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous solution [10]. It is an important technique for confirmation of formation and stability of metal nanoparticles in aqueous medium. In this method was eased by using non-toxic and simple reducing agents such as flavonoids, phenolics, terpenoids, and these AgNPs are stabilized through surface binding of capping agents, which improves stability, water solubility, and also prevent agglomeration.

### 3.3.2. Hydrophilicity and Stability of AgNPs
The phytoconstituents such as phenolics and flavonoids are present in significant amount in the leaf extract of *A. saman*. Due to the presence of flavonoid compounds in the leaf extract, the silver ion gets reduced to silver nanoparticles (Figure 1). Polyphenol compounds possess OH and C=O groups, may inactivate silver ions (Ag⁺) by chelating and additionally suppressing the superoxide driven Fenton reaction, which is believed to be the most important source of reactive oxygen species (ROS). Therefore, plants with high content of polyphenol compounds (e.g. Quercetin) are one of the best antioxidant for the biosynthesis of nanoparticle [9].

3.3.3. FT-IR Analysis
FT-IR spectrum of the freeze-dried samples was carried out to identify the feasible interactions between silver and bioactive molecules, which may be accountable for biosynthesis and stabilization of AgNPs. FT-IR analysis of algal biomass before and after bio-reduction of Ag showed the peaks for phytocomponents like alkaloids, steroids, and many of the possible bioactive compounds such as flavonoid, phenolic, terpenoids, and glycosides present in the leaf extract of *A. saman*. Due to the presence of flavonoid compounds in the leaf extract, the silver ion gets reduced to silver nanoparticles (Figure 1). Polyphenol compounds possess OH and C=O groups, may inactivate silver ions (Ag⁺) by chelating and additionally suppressing the superoxide driven Fenton reaction, which is believed to be the most important source of reactive oxygen species (ROS). Therefore, plants with high content of polyphenol compounds (e.g. Quercetin) are one of the best antioxidant for the biosynthesis of nanoparticle [9].
XRD pattern was obtained at 27.42°, 31.86°, 45.92°, 54.61°, 57.24°, 64.24°, and 76.60° for AgNPs. A corresponding to Bragg’s equation of lattice planes are observe, according to JCPDS No. 04-0783 for Ag, which could be indexed to (210), (122), (231), (142), (241), (220), (311) for AgNPs using the aqueous leaf extract of *A. saman*, and it reveals that face-centered cubic (fcc) structure of the AgNPs. The present result was good agreement with previous reports [14]. It shows that the prepared AgNPs were crystalline in nature. The small shift in the peak positions indicated the presence of strain in the crystalline structure, which is a characteristic feature of nanoparticles.

The average crystalline size of the AgNPs was estimated using Debye–Scherrer’s equation [15],

\[ D = \frac{0.94 \lambda}{\beta \cos \theta} \]

By determining the width of (122) Bragg’s reflection, the average size of the particle is 30 nm.

Flavonoids (Figure 4). Some of the peaks are present in variable intensity like, 3430, 2920, 2850, 1650, 1450, 1250, 1060, 642 cm\(^{-1}\) were shifted to few peaks at 3410, 1620, 1430, 1020, and 606 cm\(^{-1}\). A strong peak appear at 3430 cm\(^{-1}\) was obtained by the presence of alcohols and phenols with free – OH group and an absorption of the peak at 2920 cm\(^{-1}\) appearance due to –C–H group. FT-IR results exhibited that secondary molecules of amino acids have not been affected as an importance of reaction through silver ions/binding AgNPs. It was significant to understand though, that it was not just the shape and size of the nanoparticles [13].

### 3.3.4. XRD Analysis

XRD patterns were carried out to verify the crystalline nature of the AgNPs, the XRD studies are showed in Figure 5. XRD analysis revealed that intense peaks in the whole spectrum of 2θ value the ranges from 20 to 80. The
3.3.5. EDX Analysis
EDX analysis was performed to realize the percentage of Ag present in the sample. The EDX spectrum shows strong silver (62.97%) absorption peak along with various elements with their weight percentage (%) like, Carbon (15.03%), Oxygen (09.07%), Magnesium (0.52%), chlorine (12.41) in the sample (Figure 6). The results obtained that the reaction product has high purity of AgNPs. The percentage of oxygen is the smallest, with carbon and chlorine having higher percentage in the EDX spectrum analysis. The carbon and chlorine may be presented in the aqueous medium that was attached to AgNPs.

3.3.6. HR-SEM Analysis
The surface morphology of biosynthesized AgNPs are studied using FE-SEM analysis. Figure 7 shows SEM image of AgNPs that the size of synthesized AgNPs is in the range of 30–160 nm. According to the SEM image, the particle shape of plant mediated AgNPs was mostly cubic structure. This asserts the development of AgNPs by the plant extract because the aqueous leaf extract could be played as capping agent as well as reducing agent. Therefore, since it is much more significant to measure the particle size using these spectral studies.

3.3.7. In Vitro Release Study
The prepared nanoparticles were analyzed by ELISA testing method. From the results, clearly indicates that the release of silver ions from AgNPs were found to be slow and sustained for a period of 8 h. The plots of percentage release versus time in h showed that the release pattern of silver ions from AgNPs (Figure 8). Silver ions could be released for 2 h at 38.58%. Our findings confirm the efficient ability of the leaf extract of A. saman loaded AgNPs to modulate the cytokines involved in wound healing and hence controlled release of the leaf extract exhibiting effective debridement promoting wound healing.

3.3.8. Photocatalytic Activity
In this study, the dye degradation of biosynthesized AgNPs was assessed by degrading methylene blue (MB) dye in observable light irradiation. Since MB is a recalcitrant organic dye resistant to biological degradation and its presence of water present in a severe threat to aquatic life, catalytic degradation of MB is mutually a challenge in itself and a useful model for other effluents and organic pollutants [16, 17]. For the purpose of comparison, a sequence of control experiments, such as degradation in the absence of catalyst as well as degradation using commercial AgNPs and bulk Ag powder, were also performed under the same conditions. The photocatalytic property of the synthesized AgNPs was estimated by photo-degradation of methylene blue (MB), a characteristic pollutant in textile industry, when compared with AgNPs, to prepare the chemical method [18]. The Langmuir and Hinshelwood model [19] was adopted for describing the correlation between the photo-degradation reaction rates in the presence of AgNPs with respect to time. The rate of the equation can be expressed as:

\[-\frac{dC}{dt} = \frac{k_{l-H}K_{ad}C}{1 + K_{ad}C}\]
related photo-catalysts are presented in Figure 9(b). The obvious rate constant of chemical synthesized AgNPs and biosynthesized AgNPs was calculated to be 0.29627, 0.57192, 0.93001 and 1.46716/min, respectively, at 60 min. The stability of photo-catalyst in photo-catalytic activity is an important factor in the versatile applications. Therefore, the reusability of the biosynthesized AgNPs was tested by four photo-degradation cycles. To obtain the result confirms that the photo-degradation of MB using the aforementioned catalysts follows pseudo-first-order kinetics. More prominently, the biosynthesized AgNPs exhibit superior photo-catalytic activity toward the degradation of MB as contrasted to the commercially procured AgNPs and bulk silver powder. The biosynthesized AgNPs were found to possess significant photo-stability with regard to photo-catalytic degradation of MB dye (Figure 9(c)), with less than 10% decrease from its initial activity during the photo-degradation process.

### 3.4. Biological Applications

#### 3.4.1. Antibacterial Property

The results of antibacterial activity of AgNPs produced in this research are reported in Figure 10. The zone of the inhibition depends on the tested various bacteria [20]. The antibacterial effect of AgNPs on the three bacteria strain like, *S. aureus*, *E. coli*, *B. subtilis* was more potent property, when compared to *Gentamycin* (standard). A suggested idea is AgNPs after diffusion into the bacteria can inactivate their enzymes, produce hydrogen peroxide, and cause bacterial cell death [21]. The significance of antibacterial activity is definitely due to the presence of silver ions let out from AgNPs that be active as reservoirs for the Ag<sup>+</sup> bactericidal agent (Table 2).

Salopek-Sondi and Dash [22,23] was reported that AgNPs can anchor to the bacterial cell wall, penetrate
used to decrease the concentration of silver and other metal salts. The bactericidal result of metal nanoparticles has been accredited to their tiny size and quality surface the cell wall and finally leads to cell death. However, the high concentrations of silver salts restrict the use of them in present day medicine. The metal nanoparticles were used to decrease the concentration of silver and other metal salts. The bactericidal result of metal nanoparticles has been accredited to their tiny size and quality surface.

**Table 2.** Antibacterial property of AgNPs against various micro-organisms.

| Micro-organisms       | Zone of inhibition at various concentrations (μg/mL) |
|-----------------------|-------------------------------------------------------|
|                       | 10          | 20          | 30          | 40          | 50          | Control |
| *Staphylococcus aureus* | 5 ± 0.583  | 9 ± 1.241  | 11 ± 0.485 | 12 ± 0.639 | 14 ± 0.477 | 20 ± 0.244 |
| *Bacillus subtilis*    | 6 ± 0.428  | 8 ± 0.753  | 10 ± 0.599 | 13 ± 0.101 | 12 ± 0.768 | 16 ± 0.459 |
| *Escherichia coli*     | 4 ± 0.746  | 6 ± 0.427  | 8 ± 1.013  | 10 ± 1.046 | 15 ± 0.325 | 19 ± 0.564 |

Notes: $^*$ - gram-ve bacteria, $^+$ - Gram+ve bacteria. NA – no activity rendered by AgNPs against the tested micro-organisms. The results presented above indicate the efficiency of the AgNPs being active against the tested micro-organisms.
determination of the nanoparticles. The silver ions released property would be studied by ELISA method. Further, these biosynthesized AgNPs were used to study their ability to decay methylene blue dye under sunlight irradiation and the results showed potent photocatalytic property. The green synthesized AgNPs exhibited remarkable antibacterial activity. It was also obtained in potent anticancer activity with human breast cancer cells, which suggests that AgNPs could also be used as anticancer agents. From the results, these obtained AgNPs have numerous applications in the biomedical field and pharmaceutical industry as well as large scale commercial production.

Acknowledgments

The ‘INSPIRE fellowship’ is gratefully acknowledged for the award of 'DST-INSPIRE fellowship' to K. K [IF120748] and ADTWD-Scholarship to N. E.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the 'DST INSPIRE FELLOWSHIP' [grant number IF120748] and ADTWD-Scholarship.

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