Commelina maculata LEAVES EXTRACT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND EVALUATION OF ANTIMICROBIAL ACTIVITY

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ABSTRACT

There were many works have been made based on the plant and its extract mediated synthesis of nanoparticles. Metal related nanoparticles are usually synthesized by wet chemical methods, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO₃ solution through the extract of Commelina maculata leaf as it acts as a reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy and SEM analysis showed the average particle size range 15–40nm with higher density polydispersed spherical in shape. The synthesized silver nanoparticles exhibited potential antimicrobial activity against some bacteria such as Escherichia coli, Staphylococcus aureus, Bacillus subtilis and fungi as Candida albicans and Aspergillus flavus.

KEYWORDS: Commelina maculata, Silver nanoparticles, UV–Visible, FTIR, SEM, Antimicrobial activity.

1. INTRODUCTION

Nanoscience has been recognized as a new interdisciplinary science. It can be define as a whole knowledge on important properties of nano-size substances. The attach ‘nano’ designates one billionth or 10⁹ units. It is commonly believed in the perspective of nanoscience and nanotechnologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is generally established that nanoparticles are groups of atoms in the range of 1–100 nm size. Nanoparticles display completely new or enhanced properties based on definite physical appearance such as size, morphology and distribution.

Metal related nanoparticles can be synthesized by chemical, physical and biological routes; the physical approach that uses numerous methods such as condensation/evaporation and laser ablation. The chemical approach in the metal ions in solution is reduced in conditions favoring the successive development of aggregates or small metal clusters. Many metals like titanium, copper, silver, gold and iron were commonly used for the synthesis of nanoparticle. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its varied ranges of application for various sectors of industry. Currently, biosynthetic methods engaging naturally occurring reducing agents such as biological microorganism, polysaccharides such as fungus or plants and bacteria extract, i.e. green chemistry, have appeared as a modest and feasible alternative to more complex chemical and physical synthetic procedures to obtain AgNPs.

In the current eras, better progress of green synthesis of nanoparticles is expected because of its unbelievable usage in all fields of science discipline. There were various work have been created based on the plant extract intervened synthesis of nanoparticles. Many plants including Bacopa monnieri, and Catharanthus roseus used for the synthesis of AgNPs Keeping in view, in the present study to explore the novel approaches for the biosynthesis of silver nanoparticles using Commelina maculata leaf and evaluate the antimicrobial activity.

2. MATERIAL METHODS

2.1. Chemicals
All the experiments were conducted at room temperature. Chemicals used for the production of silver nanoparticles are Analytical grade silver nitrate (AgNO₃) purchased from Merck, India.

**2.2. Collection of plant materials**
The whole plant of *Commelina maculata* leaves were collected from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph’s college Trichy-Tamil Nadu. India. A Voucher specimen (TCV001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

**2.3. Preparation of leaf extract**
The dried leaves were pulverized well with mortar and pestle to make a powder. 20 grams of *Commelina maculata* leaves powder was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. The leaf extract was filtered with Whatman No. 1 filter paper after cooling. The extract was kept at 4°C for further study.

**2.4. Synthesis of Ag nanoparticles using leaf extracts**
Five ml of *Commelina maculata* leaf extract was added to 45 ml of 1 mM aqueous AgNO₃ solution in a 250 ml Erlenmeyer flask and incubated in the dark at 5hrs at room temperature. A control setup as without leaf extract also maintained. The Ag nanoparticle solution thus achieved was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the AgNPs were dried for using SEM analysis.

**2.5. UV-Vis and FTIR Spectra analysis**
The reduction of Ag⁺ ions was observed by determining the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. The formed pellet is dissolved using deionized water and filtered through whatman filter paper No: 42. This filtrate containing silver nanoparticles are used for Fourier transmission Infrared spectroscopy (FTIR).

**2.6. SEM analysis of silver nanoparticles**
Scanning electron microscopic (SEM) analysis was done using ZEISS machine. The sample were prepared as thin films on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

**2.7. Antimicrobial activity**

**2.7.1. Microorganisms**
Bacteria as *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Bacillus subtilis* (Gram positive) and fungi *Candida albicans* and *Aspergillus flavus* were the microorganisms used and they were obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

**2.7.2. Antimicrobial assay**
Antibiogram was done by disc diffusion method using herbal extracts. Petri plates were prepared by pouring 30 ml of Nutrient and Potato dextrose agar medium separately for bacteria and fungi. The test organism was inoculated on solidified agar plate with the assistance of micropipette and blowout and permitted to dry for 10 mints. The surfaces of media were inoculated with fungi/bacteria from a broth culture. A sterile cotton swab is immersed into a consistent microbial test suspension and used to equally inoculate the entire surface of the Nutrient and Potato dextrose agar plate. Briefly, inoculums comprising bacteria on Nutrient agar plates for bacteria and fungi for Potato dextrose media. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30μl of plant extract, AgNO₃ solutions, AgNPs and Standard solution as Chloramphenicol and fluconazole were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24/48 h for the bacteria and fungi at room temperature (30±1). Each sample was tested in triplicates.
3. RESULTS AND DISCUSSION

3.1. Synthesis of silver nanoparticles
The synthesis of silver nanoparticles through leaf extracts were carried out. Leaf extract is used as reducing agent as distinctive properties catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the leaf extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish-brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The appearances of yellowish-brown colour (Fig. 1) in the reaction vessels suggest the formation of silver nanoparticles (SNPs).

![AgNO₃ and AgNPs](image)

AgNO₃ : 1 mM AgNO₃ without *Commelina maculata* extract.
AgNPs : 1 mM AgNO₃ with *Commelina maculata* leaf extract after 5 hrs of incubation (Brown colour)

Figure 1 Formation of brown colour after addition of AgNO₃ indicate synthesis of AgNPs in the process of reduction of Ag⁺ to Ag nanoparticles and control (AgNO₃)

3.2. UV-Vis and FTIR Spectra analysis
It is commonly predictable that UV–Vis spectroscopy could be used to study size and shape-controlled nanoparticles in aqueous suspensions. Fig. 2 illustrate the UV-Vis spectrum noted from the reaction medium next 5 hours. The UV–vis spectra of the reaction mixture of silver nitrate solution with *Commelina maculata* leaf extract at the eaks observed at 420 nm indicate the presence of silver nanoparticles which is synthesized by *Commelina maculata* extract, the peak was raised due to the effect of surface plasmon resonance of electrons in the reaction mixture and the broadening of peak indicated that the particles are polydispersed. Appearance of this peak assigned to a surface plasmon, is well-documented for various metal nanoparticles with size ranging from 2 nm to 100 nm.
3.3. FTIR analysis of silver nanoparticles

FTIR is a commonly used method to identify the functional groups in the interactions between metal particles and biomolecules. In the present work, the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles using FTIR spectrum. The FTIR spectra of the *Commelina maculata* is given in the Fig 3. FTIR spectrum of *Commelina maculata* extract shows peak at 752, 1070, 1378, 1642, 2086 and 3430. The band peak at about 1642 cm\(^{-1}\) can be assigned for aromatic rings. The strong broad band appearing at 3430 cm\(^{-1}\) can be associated to the stretching vibrations of alcoholic and phenolic O–H. At 1070 cm\(^{-1}\) a peak is observed that could be for plant ascribed to multiplet C–O group. Therefore, from the results of FTIR analyses of extract mediated synthesized silver nanoparticles it can be concluded that some of the biological molecules of leaf extract such as flavonoids, phenols, alkaloids, glycosides, amino acids and tannins are accountable for transformation of silver ions to silver nanoparticles and its stabilization in aqueous medium. This results agreement with earlier reports\(^{13}\).

![Figure 3 FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous AgNO\(_3\) solution with *Commelina maculata* leaf extract.](image)

3.4. Scanning Electron Microscope (SEM)

The surface morphology, size and shape of the silver nanoparticles were analyzed by Scanning Electron Microscope. Fig. 4 shows the SEM image of silver nanoparticles synthesized from leaf extract. The SEM images show individual silver nanoparticles which are higher density polydispersed spherical in shape as well as number of aggregates with no defined morphology. The presences of biomolecules in the leaf extract has resulted in the synthesis of spherical silver nanoparticles and the aggregation may be due to the presence of secondary metabolites in the leaf extract. The SEM image shows the size of the silver nanoparticles ranging from 42 to 78 nm. Similar result of the silver nanoparticles size was reported by using *Coccinia grandis* leaf extract\(^8\), and by using *Allophylus serratus* Leaf\(^{14}\).
3.5. Antimicrobial activity

The SNPs of *Commelina maculata* shows highest antimicrobial activity was observed against *E. coli*, *S. aureus* and *Bacillus subtilis*. The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1 were comparable with standard antimicrobiotic viz. chloramphenicol. In this study, silver nanoparticles exhibited antimicrobial activity against *E. coli* (plate1) that was similar to that found by\(^{15}\).

The inhibitory result of Ag nanoparticles was mild in *S. aureus* and *Bacillus subtilis* (plate1) as related with other microorganisms; these results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain microbial species.

### Table 1 Anti-microbial activity of AgNPs, AgNO\(_3\), and *C. maculata* extract

<table>
<thead>
<tr>
<th>Microbial Strains</th>
<th>AgNO(_3) (30µl)</th>
<th>Plant extract (30µl)</th>
<th>AgNPs (30µl)</th>
<th>Std. (30µl)</th>
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<tr>
<td><strong>Microbial Strains</strong></td>
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<tr>
<td><em>E. coli</em> (mm)</td>
<td>3.20±0.22</td>
<td>6.80±0.54</td>
<td>11.65±0.74</td>
<td>11.20±0.86</td>
</tr>
<tr>
<td><em>S. aureus</em> (mm)</td>
<td>2.90±0.20</td>
<td>6.70±0.46</td>
<td>10.15±0.71</td>
<td>10.55±0.74</td>
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<tr>
<td><em>B. subtilis</em> (mm)</td>
<td>1.75±0.19</td>
<td>4.50±0.45</td>
<td>8.75±0.61</td>
<td>10.25±0.78</td>
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<tr>
<td><strong>Fungal Strains</strong></td>
<td></td>
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<tr>
<td><em>C. albicans</em> (mm)</td>
<td>2.10±0.15</td>
<td>3.75±0.26</td>
<td>8.25±0.62</td>
<td>7.80±0.50</td>
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<tr>
<td><em>A. flavus</em> (mm)</td>
<td>2.50±0.14</td>
<td>3.80±0.22</td>
<td>7.90±0.48</td>
<td>8.10±0.59</td>
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</table>

Values were expressed as Mean ± SD for triplicates

Microbial standard : Chloramphenicol; Fungal standard : Fluconazole.
AgNO$_3$ = Silver Nitrate; AgNPs = Silver Nanoparticles

Plate 1 shows the Antimicrobial activity of of AgNPs, AgNO$_3$ and *Commelina maculata* extract

Silver has been identified to have a disinfecting agent and has been establish in claims ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents\(^1\). In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses\(^2\). Reduction of the particle size of the materials is an efficient and reliable tool for improving their biocompatibility that can be achieved using nanotechnology.

4. CONCLUSION

Medicinal plants have therapeutically important compounds in their diverse parts. The synthesis of nanoparticles using plants depends on the nature of plant such as its phytochemical content, special adaptation, and medicinal importance. In this study, we investigated eco-friendly and cost-effective green synthesis of silver nanoparticles using leaf extract of medicinal plant *Commelina maculata*. Water soluble organic compounds present in the leaf extract was mainly responsible for synthesis of silver nanoparticles by reducing silver ions to nanosized silver particles. The UV-visible spectroscopy, FTIR and SEM studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape with size ranging between 42 and 78nm and stable. The synthesized silver nanoparticles exhibited antimicrobial activity. This finding suggests that the synthesis of AgNPs using *Commelina maculata* leaf extract could be a good source for developing green nano-medicine for the management of antimicrobial activity.

5. REFERENCES


