Antimicrobial Activity of Silver Nanoparticles from *Pithecellobium dulce*

Yamini Sudha Lakshmi**, D. Mala¹, S. Gopalakrishnan², Fouzia Banu³ and V. Brindha⁴

¹Deptartment of Biochemistry, Prof. Dhanapalan College, Chennai, India; yasula2000@yahoo.com
²PNG University of Technology, PNG
³Department of Biochemistry, JBAS College, Chennai
⁴CLRI, Chennai

**Abstract**

In this present study, the silver nanoparticles synthesized biologically from the medicinal plant *Pithecellobium dulce* were tested for its antimicrobial activity against *E.coli, S.aureus, P. aeuruginosa, and C.albicans*. The biologically synthesized nanoparticles exhibited satisfactory inhibitions against all tested microorganisms when used at different concentrations viz. 1 mM, 3 mM and 5 mM; 3 mM (20 mg/500 µl distilled water) offered the highest sensitivity. Such green-synthesized antibacterial agents locally destroy bacteria, without being toxic to the surrounding tissue.

**Keywords:** *E.coli, Calbicans, Paeuruginosa, Pithecellobium dulce, S.aureus*

1. Introduction

Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth, without being in general toxic to surrounding tissue. Most current antibacterial agents are chemically modified natural compounds¹, for instance, β-lactams (like penicillins), cephalosporins or carbapenems².

Disease causing microbes that have become resistant to drug therapy are an increasing public health problem. Therefore there is an urgent need to develop new bactericides. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes³.

Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non-toxic to environmental routes. Green synthesis offers improvement over synthetic, chemicals and micro-organisms methods as it is cost effective, environmentally friendly and can easily be scaled up for large scale synthesis. The methods used for the synthesis of silver nanoparticles and toxic chemicals are used for the reduction process of substances such as citrates, NaBH₄, or ascorbates. Recently, green bio-reduction methods for the synthesis of silver nanoparticles were adapted by many researchers using plant extracts such as *Macrotyloma uniflorum*⁴, *Anacardium Mushroom extract*⁵, *Coleus amboinicus lour*⁶, *Medicago sativa*⁷, *Chenopodium murale*⁸, *Emblica Officinalis* Fruit Extract⁹, *Eucalyptus hybrid*¹¹, etc.

In the present study an attempt was made to study the antimicrobial activity of Silver nanoparticles synthesized from the medicinal plant *Pithecellobium dulce* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeuruginosa and Candida albicans.*

*Author for correspondence*
2. Materials and Methods

2.1 Preparation of Silver Nanoparticle Crude Extract Discs

Sterile Whatman No.1 paper was punched into 5mm diameter disc sizes. The Whatman discs were placed in MacCartney bottles and sterilized in an autoclave at 120 °C for 15 min. The bottle was transferred into a hot air oven at 60 °C to dry for 30 minutes. 30 microlitres of 3 mM & 5 mM silver nanoparticles prepared from *Pithecellobium dulce*, [20 mg in 500 microlitres of sterile distilled water] by using a mixer and suspended on the punch prepared discs by applying 10 µl inoculation each time followed by air drying and stored in sterile containers.

2.2 Antimicrobial activity

The microorganisms used for the study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231). Mueller Hinton agar (HI media) was used for the performance of the antimicrobial assay. Gentamycin (10 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg) and Ampicillin (10 µg) were used as controls for the bacteria’s. Wells were made (6 mm diameter) by using an autoclave sterilized metallic borer. Well isolated fresh colonies of the microorganisms were used to prepare inoculum suspension equivalent to 0.5 Standard McFarland Turbidity (which is $1.5 \times 10^4$ Colony Forming Units per ml); microbes were inoculated and incubated at 37 °C for 24 hours. After 24 hours the media were examined for inhibition zones and results were recorded in millimeter.

When the antimicrobial activity of the 20 mg/500 µl of the 3 mM and 5 mM concentration of silver nanoparticles were compared it was significantly proved that the 3 mM concentration of the silver nanoparticles were more sensitive towards the microorganisms than that of 5 mM concentration of the silver nanoparticles. Additionally, when the concentration of the nanoparticles was increased (i.e. from 50 µl to 100 µl) the sensitivity towards the microorganisms was also increased. It was found out that the higher the concentration of crude extract, the higher the diameter of the inhibition zone.

From the above results it was clear that the 20 mg (suspended in 500 µl distilled water) of the 3 mM concentration of the silver nanoparticle gave the highest sensitivity.

However, when the silver nanoparticles were impregnated on the discs, the sensitivity recorded was poor as compared (see Table 1) to that of the ones inserted into the well. This could be due to the proper distribution of the particles throughout the media when inoculated in the medium well. And also, the diameter of the discs was

<table>
<thead>
<tr>
<th>Name of microbes</th>
<th>3 mM (in disc) 30 µl</th>
<th>5 mM (in disc) 30 µl</th>
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<tbody>
<tr>
<td>E.coli</td>
<td>11 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>S.aureus</td>
<td>10 mm</td>
<td>9 mm</td>
</tr>
<tr>
<td>P.aeuruginosa</td>
<td>11 mm</td>
<td>10 mm</td>
</tr>
<tr>
<td>C.albicans</td>
<td>12 mm</td>
<td>11 mm</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Name of microbes</th>
<th>3 mM (in well) 20 mg in 500 µl distilled water</th>
<th>5 mM (in well) 20 mg in 500 µl distilled water</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>50 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>E.coli</td>
<td>13 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>S.aureus</td>
<td>13 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>P.aeuruginosa</td>
<td>15 mm</td>
<td>16 mm</td>
</tr>
<tr>
<td>C.albicans</td>
<td>16 mm</td>
<td>18 mm</td>
</tr>
</tbody>
</table>
5 mm, as a 6 mm puncher was not available, now since the diameter of the disc is reduced by 1 mm; it is likely to have affected the results.

Additionally, when the effect of the particles against the bacteria’s (i.e. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and the fungus (*Candida albicans*), was evaluated, they seem to have a relatively higher zone of inhibition towards the fungus than the bacteria’s study revealed that the fungus *Candida albicans* was found to be resistant when tested by the leaf of *Pithecellobium dulce* crude extract.

The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulphydryl) groups, although other target sites remain a possibility. Silver was also proposed to act by binding to key functional groups of enzymes. Bacterial cells increased in size, and the cytoplasmic membrane, are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics because the metal attacks a broad range of targets in the organisms which means that they would have to develop host mutations simultaneously to protect themselves. Cytoplasmic contents and outer cell layers all exhibited structural abnormalities. Finally, silver ions interact with nucleic acids; they interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of their lethal action is unclear. It was shown in the present study that the antimicrobial activity of the silver nanoparticles synthesized from *Pithecellobium dulce* had sensitivity against the microbial strains *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. This was also evidenced by the work of Goodsell, which used *Pleurotus sajor caju* silver nanoparticles.

### 3. Conclusion

The silver nanoparticles prepared biologically from the plant *Pithecellobium dulce* developed sensitivity against the microbial strains *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The 100 µl of 20 mg/500 µl of the 3 mM silver nanoparticle showed the highest sensitivity among the different concentrations used. This antibacterial property can be used in textile industry, water disinfection, medicine, and food packaging.

### 4. References