**In vitro Antibacterial Activity of Green Synthesized Silver Nanoparticles Using Jamun Extract Against Multiple Drug Resistant Bacteria**

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Abstract: Objective: To determine bacteriocidal property of green synthesized silver nanoparticles using jamun extract as reducing agent against multiple drug resistant bacteria (MDR) viz. MRSA (methillin resistant Staphylococcus aureus), ampicillin-resistant Escherichia coli, erythromycin-resistant Streptococcus pyogenes. Methods: silver nanoparticles were synthesized through jamun extract and characterized using UV-VIS spectroscopy, SEM, TEM techniques. The antibacterity activity assays were done against three bacterial pathogens by well diffusion method. Results: The diameter of silver nanoparticles was predominantly found within the range 30-100 nm. The absorption peak at 422 nm broadens with increase in time indicating polydispersity nature of the nanoparticles. Conclusions: The present findings suggest that green synthesis route is simple yet quite effective method for obtaining those silver nanoparticles that can be used as an alternative antibacterial agent against diseases caused by multiple drug resistant pathogens.

Keywords: Silver nanoparticles • Green synthesis • Multiple drug resistant

**INTRODUCTION**

In the present scenario, pharmaceutical and biomedical sectors are facing the challenges of continuous increase in the multidrug-resistant (MDR) human pathogenic microbes. Re-emergence of MDR microbes is facilitated by drug and/or antibiotic resistance, which is acquired way of microbes for their survival and multiplication in uncomfortable environments. MDR bacterial infections lead to significant increase in mortality, morbidity and cost of prolonged treatments. Therefore, development, modification or searching the antimicrobial compounds having bactericidal potential against MDR bacteria is a priority area of research. Silver in the form of various compounds and bhasmas have been used in Ayurveda to treat several pathogenic bacteria since time immemorial. As several pathogenic bacteria are developing antibiotic resistance, silver nanoparticles are the new hope to treat them. Silver nanoparticles having size in the range of 10-100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria. The bactericidal activity of silver nanoparticles against the pathogenic, MDR as well as multidrug-susceptible strains of bacteria was studied by many scientists and it was proved that the silver nanoparticles are the powerful weapons against the MDR bacteria.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions [1] chemical and photochemical reactions in reverse micelles [2], thermal decomposition of silver compounds [3], radiation assisted [4], electrochemical [5], sonochemical [6], microwave assisted process [7] and recently via green chemistry route [8-10]. Green synthesis offer better manipulation, control over crystal growth and their stabilization.

Here in the current work we wish to reported the synthesis of green silver nanoparticles using the leaf extract of the plant - *Syzygium cumini* (common name- Jambul, Jamu koli). Aqueous silver nitrate solution, after reacting with jamun leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles. The rate of nanoparticle synthesis was very high, which justifies use of plants over microorganisms in the biosynthesis of metal nanoparticles through greener and safer methods. In the subsequent sections we have
described the synthesis of silver nanoparticles based upon the change in color, change in pH, change in absorbance and the particle size formed after reduction.

**Plant Description**  
*Syzygium cumini*  
*Family:* Myrtaceae

**Common Name:** Jambul, Jamu koli  
It has a high source in vitamin A and vitamin C, thiamine (vit-B1) 1%, riboflavin (vit-B2) 1%, Niacin (vit-B3) 2%, plantothenic acid (B5), Vit-B6 3%, ascorbic acid (vit-C) 17%. Medicinally all parts of plant seeds, leaf, bark are used. Properties like antidiabetes, antibacterial, astringent, digestive, diuretic, anthelmintic and is considered useful for throat problems, stomachic, carminative, antiscorbutic and diuretic are being reported (Figure 1).

**MATERIAL AND METHODS**

**Reagents and Chemicals:** 0.001 M Silver Nitrate was obtained from Sigma Aldrich. Freshly prepared triple distilled water was used throughout the experiment.

**Collection of Extracts:** Jamun leaves were collected from the local region. They were washed and cleaned with triple distilled water and dried with water absorbent paper. Then it was cut into small pieces with an ethanol sterilized knife and crushed with mortar and pestle dispensed in 10 ml of sterile distilled water and heated for 2-3 minutes at 70-80°C. The extract was then filtered using Whatman No.1 filter paper. The filtrate was collected in a clean and dried conical flask by standard sterilized filtration method and was stored at 4°C.

**Synthesis of Zero Valant Silver Nanoparticles:** During the synthesis of Silver Nanoparticles both the precursor and the reducing agent were mixed in a clean sterilized flask in 1:1 proportion. For the reduction of Ag ions, 5ml of filtered plant extract was mixed to 5 ml of freshly prepared 0.001 M aqueous of AgNO3 solution with constant stirring at 50-600°C. The Silver Nanoparticles so prepared were stabilized by adding 1% of chitosan and 1% of PVA.

**UV-Vis Spectra Analysis:** The reduction of pure Ag ions to Ag0 was monitored by measuring the UV-Vis spectrum by sampling of aliquots (0.3 ml) of Ag Nanoparticle solution diluting the sample in 3 ml distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 at the range of 200-600 nm and observed the absorption peaks at 400-440 nm regions due to the excitation of surface plasmon vibrations in the AgNPs solution, which are identical to the characteristics UV-visible spectrum of metallic Iron and it was recorded.

**pH Analysis:** 1 mM aqueous silver nitrate (AgNO3) solution shows 3.8 pH, there is concerned change in pH was determined of silver nanoparticle synthesis using extracts of plant and spices, which was determined using Digital pH meter Systronics.

**SEM Analysis:** Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared 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 film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

**TEM Analysis:** After bioreduction, the mixture were sampled for TEM observation on H-600 Electron Microscope (Hitachi) at a voltage of 120kV.

**Collection of MDR Strains:** Three strains under MDR category such as MRSA (methillin resistant Staphylococcus aureus), ampicillin-resistant Escherichia coli, erythromycin-resistant Streptococcus pyogenes were collected from SCB pathology laboratory, Cuttack.

**Antibacterial Assay:** The antibacterial activity of the green synthesized silver nanoparticles was performed by using well diffusion method. About 20 mL of sterile molten
Mueller Hinton agar was poured into the sterile petriplates. Triplicates plates were swabbed with the overnight culture (10^8 cells/mL) of pathogenic bacteria viz. *E. coli*, *S. aureus*, *S. pyogenes*. The solid medium was gently punctured with the help of cork borer to make a well. Finally, the nanoparticle samples (50 g/ml) were added from the stock into each well and incubated for 24 hr at (37±2)°C. After 24 hr, the zone of inhibition was measured and expressed as millimeter in diameter.

**RESULTS AND DISCUSSION**

**Synthesis of Silver Nanoparticles:** Jamun leaf extract is used to produce silver nanoparticles in this experiment. Ag ions were reduced into Ag°nanoparticles when plant extract is mixed with AgNO₃ solution in 1:1 ratio. Reduction is followed by an immediate change in color from pale brownish to dark grayish and change in pH of the solution.

<table>
<thead>
<tr>
<th>Table 1: Change in color of the solution during silver nanoparticle synthesis</th>
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<tbody>
<tr>
<td><strong>Sr. No</strong></td>
</tr>
<tr>
<td>1.</td>
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<tr>
<td>2.</td>
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Color intensity: - += Light color, ++= Dark color, +++= Very dark color

<table>
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<tr>
<th>Table 2: Change in pH during silver nanoparticle synthesis</th>
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<tr>
<td><strong>Plant Extract</strong></td>
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<tr>
<td>-----------------------------------------------------------</td>
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<tr>
<td><strong>Binomial Name</strong></td>
</tr>
<tr>
<td><em>Syzygium cumini</em></td>
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Result: - += Positive, -= Negative.

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<th>Table 3: Antibacterial tests</th>
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<tr>
<td><strong>Inhibition zone (mm)</strong></td>
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<tr>
<td><strong>Jamun extract</strong></td>
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<tr>
<td><strong>Sl no.</strong></td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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**Fig 2:** a) Jamun silver nanoparticle SPR at 422 nm, b) Tube A- silver nitrate, Tube B - Extract, Tube C- Jamun silver nanoparticle solution.

**Fig 3:** SEM Image of Ag nanoparticles

**Fig 4:** TEM Image of Ag nanoparticles

**pH Analysis:** On mixing the plant extract with the aqueous changed the color of the solution immediately and reducing the pH, which may be an indication of formation silver nanoparticles. In this experiment it was observed that the pH changed from high acidic to low acidic.
UV Visible Spectroscopy and Color Change for the Green Synthesized Silver Nanoparticles: The UV visible spectroscopy of the synthesized nanoparticles were in the range of 400-440 nm. Jamun leaves extract was shown to synthesize the silver nanoparticles by the indication of suitable surface Plasmon resonance (SPR) with high band intensities and peaks under visible spectrum at 422 nm (Figure 2).

SEM Analysis: The SEM image showed relatively spherical shape nanoparticle formed with diameter range 30-40 nm (Fig.3).

TEM Analysis: From the given TEM images, it is concluded that green synthesized silver nanoparticles are of irregular shapes which varies in size from 30-100 nm. (average particle size is 50 nm).

Antibacterial Activity Analysis: The antibacterial tests provide evidence that the silver nanoparticles formed due to reduction by grape fruit extract show sufficient antimicrobial resistance against bacterial pathogens (Table 3).

CONCLUSION

It has been demonstrated that extract is capable of producing silver nanoparticles that shows good stability in solution, under the UV-Visible wavelength nanoparticles shown quiet good surface plasmon resonance behavior. Silver Nitrate with reducing agent i.e. jamun Leaves extract has shown a remarkable color change with concerned change in pH of solution. Success of such a rapid time scale for synthesis of metallic nanoparticles is an alternative to chemical synthesis protocols and low cost reductant for synthesizing iron nanoparticles.

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REFERENCES