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# "Characterization and Green Synthesis of Silver Nanoparticles from *Plumeria* Leaves Extracts: Study of Their Antibacterial Activity".

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***Abstract****: In the present work, the stable silver nanoparticles were synthesized by the bioreduction method. Aqueous leaf extracts of the Plumeria plant was used as reducing and as capping agent. The color change in reaction mixture from bright green to dark brown color was observed which indication of the reduction of Silver ions into Silver nanoparticles. The formation of silver nanoparticles (AgNPs) was characterized by UV-vis spectroscopy, FT-IR, SEM and XRD studies. The synthesized AgNPs exhibited good Antibacterial potential against gram positive and gram negative bacteria.*

***Keywords:*** *Plumeria, Silver nanoparticles, UV-vis, XRD, SEM, Anti bacterial activity.*

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## I. Introduction

Nanotechnology is a branch of science and engineering involving the synthesis of nanoparticles at the nanoscale, i.e. 1 to 100 nm. Nanoparticles possess high surface to volume ratio due to their small size, which gives very distinctive features to the nanoparticles. The study about nanoscience and nanotechnology provides the well developed application of exceptionally miniature things and be capable of the encroachment of all the fields of scientific research and development like Physics, Chemistry, Materials and Metallurgy engineering, Biology and also in Biotechnology [1, 2]. At present, there is an emergent need to develop environmentally benevolent nanoparticles synthesis routes, which can be proceeded by biological method instead of chemical methods. The use of ecologically beneficial materials like plant leaf extracts, bacterial cell extracts, fungi and enzymes for the synthesis of nanoparticles proposes abundant benefits in terms of eco-friendliness and compatibility for a wide range of pharmaceuticals. Accordingly, the researchers in the field of nanoparticles synthesis and assembly have turned to biological systems for inspiration [3]. Currently, the use of green synthesis methods for the production of engineered nanomaterials in both industrial application and the scientific research has achieved a massive amount of interests [4]. Green synthesis method is beneficial over other methods which are implemented for the synthesis of nanoparticles. Green synthesis methods are eco-friendly approach and compatible for pharmaceutical and other biomedical applications, as the toxic chemicals are not used in these methods [5]. While chemical synthetic procedures can lead to the generation of toxic chemical by-products or require high temperatures and/or pressure, biosynthesis of nanoparticles using plant extracts provides a facile and 'green' method of nanoparticle synthesis.[6,7] Silver Particles are of more interest in their colloidal perpetrations because of their distinctive properties, like chemical stability, conductivity, catalytic and antibacterial activities [8]. Silver in different forms and as nanoparticles have been used in medicine for dental materials, wound treatment, coating on stainless steel materials, water purification and in sunscreen lotions [9]. Silver has long been recognized as an antiseptic and anti-biotic since is having an inhibitory effect towards many microorganisms [10]. Many researchers are have used variety of plants for the synthesis of silver nanoparticles. The following plants and their extracts have been used for green synthesis; leaves extract of castor oil (*Ricinus Communis)*, khat (*Catha Edulis*) and sun flower (Helianthus Annuus)[11], O*cimum sanctum*[12], *Aspergillus oryzae*[13], *Crinum asiaticum*[14], Azadirachta indica aqueous [15,16], Bamboo [17], *Dodonaea viscosa* and *Capparis deciduas*[18], *Bryophyllum pinnatum*[19] and fruit extract of Andean blackberry [20].

*Plumeria* is a genus of flowering plants in the family, Apocynaceae. *Plumeria* is related to the Oleander, Nerium oleander, and both possess an irritant, rather similar to that Euphorbia. The leaves of *Plumeria* are narrow and corrugated dark-green color. The various species of *Plumeria* are known to have medicinal properties and have a long history of use by indigenous and tribal people in India. The medicinal

value of this *Plumeria* species in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita [21]. In the present work, the green synthesis of AgNPs is attempted with leaves extracts of *Plumeria.* The AgNPs obtained are characterized and evaluated for their antibacterial activities.

**Fig. 1** *Plumeria.*

## II. Experimental

### A. Materials

Fresh leaves of *Plumeria* plant free from diseases were collected from the campus garden of Shridevi Institute of Engineering and Technology, Sira Road, Tumakuru, Karnataka, India. *Staphylococus aureus, Klebsiella aerogenes, Pseudomonas aeruginosa and Escherichia coli* bacterial strains were collected from department of microbiology, Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India.The nutrient media used was supplied by Hi-Media Laboratories. $AgNO_3$ and KCL were procured from Merck, Mumbai, India.

### B. Methods

**1) Preparation of leaves extracts**:

For the synthesis of silver nanaoparticles, the leaves of *Plumeria* were washed thoroughly with tap water to remove the dust and dirt particles and then washed with double distilled water. 20 g of chopped leaves were added to 100 ml double distilled water and stirred at 60°c for 30 min on heating mantle. After boiling, the mixture was cooled for 20 min and filtered through Whatman filter paper No.1. The collected leaves extracts (bright green color) was used for reducing and as capping agent in AgNPs synthesis.

**2) Synthesis of Silver Nanoparticles using *Plumeria* leaves extracts:**

5 ml of *Plumeria* leaves extracts were added to the 45 ml of 5mM $AgNO_3$ solution at ambient temperature and stirred continuously for 15 min using magnetic stirrer. Slow reduction takes place and kept for 24 hours to obtain the color change for bio-reduction process. After 24 h bright green color changed to dark brown color which indicates the formation of AgNPs (Fig.2) The AgNPs obtained from the solution was purified by repeated centrifugation at 8,000 rpm for 15 min using Remi cooling centrifuge C-24. The AgNPs obtained were dried and stored for further analysis.


| | | |
|---|---|---|
| **A**- Leaves extracts | **B**-5mM $AgNO_3$solution | **C**- Leaves extracts + $AgNO_3$ after 24 hour |

**Fig. 2** Formation of AgNPs.

**3) Analysis of Silver nanoparticles:**

**a) Phytochemical analysis:**

The leaves extracts of *Plumeria* were assessed for the qualitative determination of chemical constituents i.e. alkaloids, saponins, phenols, flavonoids, tannins, terpenoid and glycosides by applying standard procedures.

**b) UV-Vis Spectra analysis:**

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1ml of distilled water used as blank.The sample was analysed by UV-Vis spectrophotometry (model Shimadzu UV) for its maximum absorbance v/s wavelength to confirm the formation of AgNPs.

**c) Fourier Transform Infra-Red spectroscopy (FT-IR) analysis:**

The sample was mixed with KCl and a thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infra Red [FTIR] for the analysis of the nanoparticles. The FTIR measurement sample was recorded in the range of 400-4000$cm^{-1}$ using Nicolet Avatar model. It gives information on the rotations and vibrations modes were identified and purposed to determined the distinct functional groups present.

**d) X-Ray diffraction analysis:** The reduced AgNPs powder was coated on a glass substrate and the X-ray diffraction measurement were carried out by using a powder X-ray (PAN analytical BV model) instrument operating at a voltage of 40kV and current of 30mA. The output was recorded in the form of a graph with 2θ on x-axis and then intensity on y-axis. The crystallite average size of particle was calculated by using the Debye-Scherrer formula.

$D=k\lambda/\beta\cos\theta$,

where λ is wavelength, D is particle diameter size, β is the full width half maximum, k is a constant (value 0.9) and θ is Braggs diffraction angle.

**e) Scanning Electron Microscopy analysis:**

After the preparation of the nanoparticles, the particle size and their morphological distribution were assessed with Scanning Electron Microscopy (SEM). A drop of aqueous solution containing purified silver nanoparticles obtained after repetitive centrifugation was placed on the carbon coated copper grids and dried under infrared lamp for characterization of their morphology using FEI Quanta 200 Scanning electron microscope at accelerating voltage of 20 keV.

**f) Antimicrobial activity of silver nanoparticles:**

The antibacterial activity of AgNPs produced by *Plumeria* leaves extracts were evaluated by the disc diffusion method. *Klebsiella aerogenes, Staphylococus aureus, Pseudomonas aeruginosa and E-coli* bacterial strains. The bacterial strains were developed in nutrient broth (NB) media for 24 h at 37°c and 1 ml of each broth culture was spread over the nutrient agar media. 5 mm sterilized filter paper discs were dipped in synthesized Silver nanoparticles suspension (10µg/ml), double distilled water as negative control, Taxim (1µg/ml) as standard and leaves extract was placed over the agar plates and incubated for 24 h at ambient temperature.

## III. Results And Discussion

**a) Phytochemical analysis**

The results of phytochemical analysis of *Plumeria* leaves extracts are presented in table.1 and flavonoids, saponins, alkaloids, phenols and glycosides are present.

**Table.1** Phytochemical analysis of *Plumeria* leaves extracts

| S.No. | Phytochemicals | Leaves extract |
|---|---|---|
| **1** | Flavonoids | ++ |
| **2** | Alkaloids | +++ |
| **3** | Phenols | ++ |
| **4** | Tannins | －－ |
| **5** | Glycosides | ++ |
| **6** | Saponins | +++ |
| **7** | Terpenoid | － |

**+: Confirms, －: Absent.**

**b) UV-Vis-spectroscopy analysis:**

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Plumeria* plant leaves extracts have been studied by the UV-Vis spectroscopy and found that UV-Vis spectrograph of the colloidal solution of AgNPs has been recorded as a function of time by using a quartz cuvette with water as reference. Maximum absorbance peak was observed at 451 nm indicating that the formation of silver nanoparticles as a result of reduction of $Ag^+$ ions present in the aqueous $AgNO_3$ solution (Fig.3).

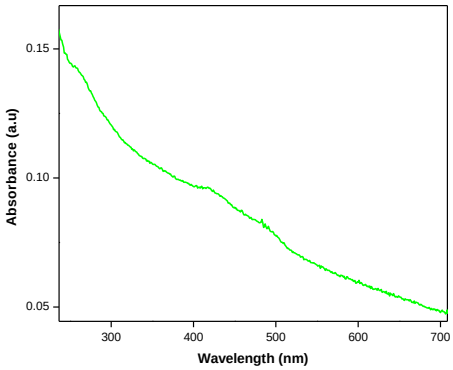



**Fig.3** UV-vis spectrum of AgNPs synthesized by *Plumeria* leaves extract.

**c) FT-IR analysis:**

Fourier transform-Infrared (FT-IR) analysis was performed to identify the possible biomolecules responsible for the reduction of the $Ag^+$ ions and capping of the reduced AgNPs synthesized using *Plumeria* leaves extract. The strong IR bonds were observed at 3,703, 3381, 2,925, 2333, 1,618, 1,387, 1,070, and 601 $cm^{-1}$. The bands which appeared at 3,703 and 2,922 $cm^{-1}$ corresponding to N-H, –OH stretching and aliphatic –C-H stretching, respectively. The bands at 2,333 and 1,618 $cm^{-1}$ are due to the $CO_2$ and C=C stretching, respectively. The IR bands observed at 1,387 and 1,070 $cm^{-1}$ may be ascribed to –C-O and –C-0-C stretching modes, respectively. The strong bands recorded at 601 $cm^{-1}$ in the spectra of the synthesized material were assigned to C-H bending peak may be raised due to the reduction of $AgNO_3$ to Ag nanoparticles (Fig. 4).

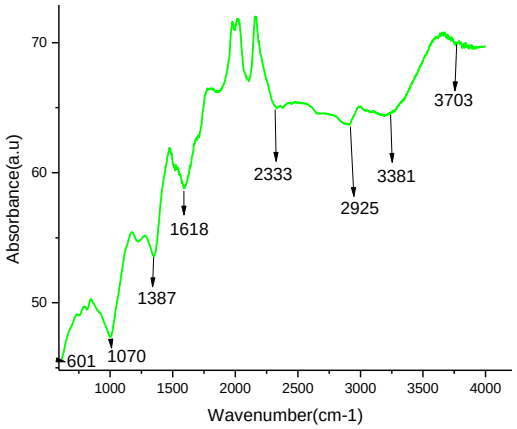



**Fig.4** IR spectra of AgNPs synthesized using *Plumeria* leaves extract.

**d) X-ray diffraction:**

X-ray diffraction (XRD) pattern was recorded for the synthesized AgNPs (Fig. 5), shows a number of Bragg reflections corresponding to (111), (200), (220) and (311) sets of lattice planes are observed. Which may be indexed based on the structure of Ag. The diffraction peaks at 2θ= 38°, 44°, 64° and 77° were indexed with the planes (111), (200), (220) and (311) for the fcc lattice of obtained silver as per the Joint Committee on Powder Diffraction Standards (JCPDS) card no. 04-783 was matched with database. The average size (D) of

synthesized Silver nanoparticles was found to be 41.7 nm as calculated by using Debye-Scherer formula. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

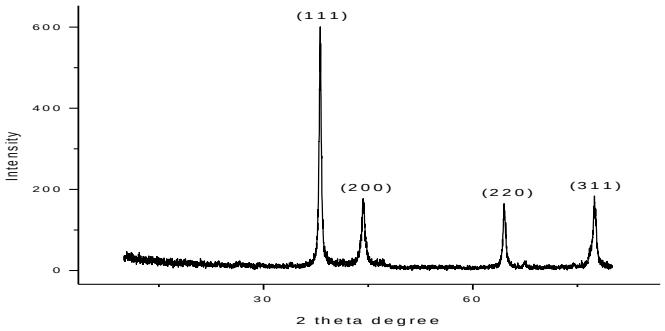



**Fig.5** XRD pattern of synthesized AgNPs

**e) Scanning Electron Microscopy (SEM) analysis:**

SEM analysis shows the uniformly distributed AgNPs on the surface of the cells. However, it does not indicate that all the NPs are bound to the surface of the cells, because those dispersing in the solution may also deposit onto the surface of the cells. The SEM image (Fig. 6) has shown separate AgNPs as well as particle agglomeration. The SEM study indicates that the particle size is irregular and shape of the particles is spherical in morphology of size ranging from 36 to 47 nm with an average size of 41.90 nm.

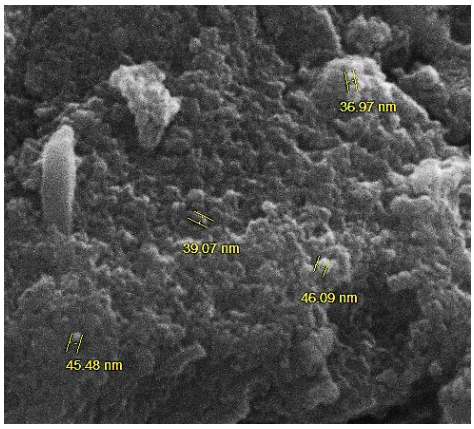
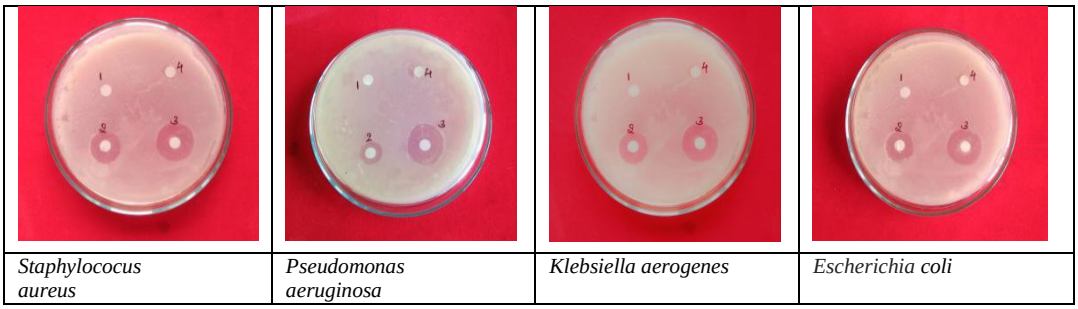



**Fig. 6** SEM images of synthesized AgNPs from *Plumeria* leaves extracts.

**f) Antibacterial Assay**

The synthesized AgNPs by the leaves extracts of *Plumeria* have a significant antibacterial activity against *Staphylococus aureus* followed by *Pseudomonas aeruginosa, Klebsiella aerogenes* and *E-coli* (Fig.7; Table. 2).



| *Staphylococus aureus* | *Pseudomonas aeruginosa* | *Klebsiella aerogenes* | *Escherichia coli* |
|---|---|---|---|

**Fig. 7** Antibacterial activity of AgNPs synthesized by leaves extracts of *Plumeria*

**Table.**2 Antibacterial Zone of Inhibition.

| **Zone of Inhibition (in mm)** | | | | | |
|---|---|---|---|---|---|
| S.No | Strains | (1) Control | (2) Standard | (3) AgNPs | (4) Leaves Extract |
| 1 | *Escherichia coli* | _ | 19mm | 27mm | _ |
| 2 | *Pseudomonas aeruginosa* | _ | 15mm | 27mm | _ |
| 3 | *Klebsiellaaerogenes* | _ | 18mm | 26mm | _ |
| 4 | *Staphylococus aureus* | _ | 19mm | 28mm | _ |

Control - double distilled water, AgNPs - Silver Nanoparticles, Standard -Taxim, Leaves Extract - *Plumeria* leaves extracts.

## IV. Conclusion

It is concluded that the leaves extracts of *Plumeria* is capable of producing silver nanoparticles extracellular and are quite stable in solution. From the XRD data studies the calculated average size of synthesized silver nanoparticles was found to be 41.7 nm. The SEM studies confirm the particle size is irregular and shape of the particles was found to be spherical in morphology with an average size of 41.90 nm. The synthesized AgNPs by *Plumeria* leaves extracts has shown efficient antimicrobial activities against *Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa* and *Staphylococus aureus*.

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