

## Plumbago Zeylanica's Biosynthesis of Silver Nanoparticles: Characterization and Sustainable Environmental Uses

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**Abstract:** Due to *Plumbago zeylanica* own ecological features and environmental management applications, biological synthesis of silver nanoparticles (AgNPs) has become increasingly intriguing. Moreover, the green synthesis of AgNPs using *Plumbago zeylanica* aqueous extract—being a medicinal plant rich with bioactive compound—has been the main focus in this study. The reduction of silver ion into nanoparticle was carried out with the aid of plant extract as stabilizing and reducing agent. There are produced AgNPs without the involvement of any toxic chemical agent. The morphology, size, and crystal structure detection for synthesized AgNPs were characterized using fully automated X-ray diffraction (XRD), transmission electron microscopy (TEM), and UV-Vis spectroscopy. The use of said AgNPs in environmental remediation, especially water treatment, is investigated. The bactericidal properties of AgNPs were tested against waterborne pathogens indicating that the formation of microbial biofilms can be prevented effectively since the number of viable microorganisms was significantly reduced. In addition, the similarity between the enzymes in various living systems resulted in high biological activity and function through a catalytic process as an aid to degrade organic pollutants which are inclusive of dyes and heavy metals. Furthermore, this green methodology for synthesizing AgNPs presents an economically and ecologically facile route for their large-scale production. Thus, *Plumbago zeylanica*-fabricated AgNPs can potentially help in mitigating environmental pollution; thus providing new strategies for pollution control measures and water treatment.

**Keywords:** Antimicrobial Activity, Environmental Applications, Green Synthesis, Nanotechnology, Photocatalytic Activity, *Plumbago zeylanica*, Silver Nanoparticles (AgNPs)

## Introduction

Nanotechnology has become a powerful means of change in the environmental field, where nanomaterials have unique physicochemical properties such as their size and structure. With their superb antibacterial, catalytic, and surface-enhancing capabilities, silver nanoparticles (AgNPs) are the ones that have attracted the most attention and they are therefore excellent choices for water and environmental cleanup. However, AgNP synthesis methods are very toxic and the energy produced is energy-intensive, which has a negative impact on the environment and human health. As a more environmentally sustainable substitute, scientists have taken to the use of green synthesis methods, which involve plant extracts acting as stabilizing and reducing agents [3]. One of the recognized applications of *Plumbago zeylanica*, called Peolii, is its use as an antibacterial, anti-inflammatory, and anti-fungal substance in traditional medicare [4]. The bioactive chemicals among the *P. Zeylanica* that include Flavonoids, alkaloids, and phenolic acids are the green chemicals that the bioengineering of AgNPs through this plant may be feasible. These substances are believed to have two functions: they stabilize the nanoparticles to hinder their aggregation and the silver ions ( $Ag^+$ ) are transformed into AgNPs [6]. The synthesis of silver nano-materials (AgNPs), it can be illustrated by utilizing plant extracts, which are known as a green technique in the water treatment area and it allows environmental concerns to be addressed. The statement was made in the project that can be found in the literature, that atNEPs are not only antibacterial but also antiviral agents because of their broad-spectrum antibacterial activity [7]. It was discovered that while AgNPs act as catalysts for the conversion of common chemical pollutants such as heavy metals and dyes, they were also broken down. The study also highlights the potential of these nanomaterials in the treatment of business wastewater that has frequent contamination issues. The nanoparticles made from *P. Zeylanica* could be a wonderful option and the application of the same in the synthesis of AgNPs could not be said more for the environmental remediation initiatives. The work being carried out is on the production of environmentally friendly AgNPs by the Synthesis of *P. Zeylanica* and their application in water treatment, such as the degradation of pollutants and microbial disinfection will be investigated.



## 1- Materials and Method

### Materials

*Plumbago zeylanica* fresh leaves were plucked from a nearby botanical garden or medicinal plants site. After washing the leaves with distilled water to remove any dirt or outside contaminants, they were left to dry for a few days at room temperature in the shade. The leaves were dried and then ground into a powder for extraction.

Now 150 millimeters of distilled water mixed with around 16 grams of dry leaf powder were the components needed for the aqueous extract. During the process of the reduction of silver ions, bioactive chemicals responsible for the tingling effect were removed. The mixture was boiled for 60 minutes at a temperature between 60 and 80°C to enhance the plant extraction and obtain the organic compounds[3]. After the extract has reached the ambient temperature, it was passed through a Whatman No. 1 filter paper to eliminate plant debris and achieve a clear solution[5].

In order to create the silver nanoparticles, 90 mL of a 1 mM silver nitrate ( $\text{AgNO}_3$ ) solution was mixed continuously with 10 mL of the *Plumbago zeylanica* leaf extract. The color of the reaction mixture gradually changed from pale yellow to brown as it was maintained at room temperature, signifying the creation of silver nanoparticles [3]. The reaction was run for a whole day in order to finish the reduction.

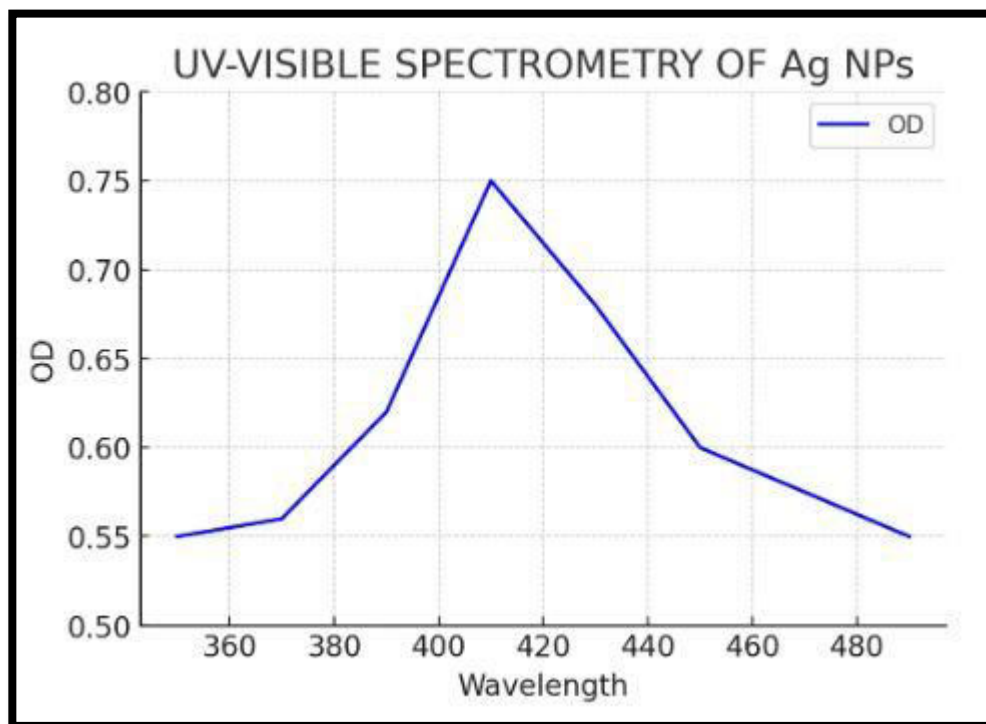


The observed color shifting due to the surface plasmon resonance (SPR) of the silver nanoparticles, pointed out the existence of their creation.

## 2- Characterization of Silver Nanoparticles

### a) UV-Vis Spectroscopy

UV-Vis spectrophotometer, was used in the process of synthesizing AgNPs where the absorbance data were tracked for the verification of the nanoparticles created [7]. The creation of nanoparticles was validated through the scanning of the sample in the 300–600 nm range that was also expected to have the usual AgNP surface plasmon resonance peak at 420–450 nm.



#### b) Fourier Transform Infrared Spectroscopy (FTIR)

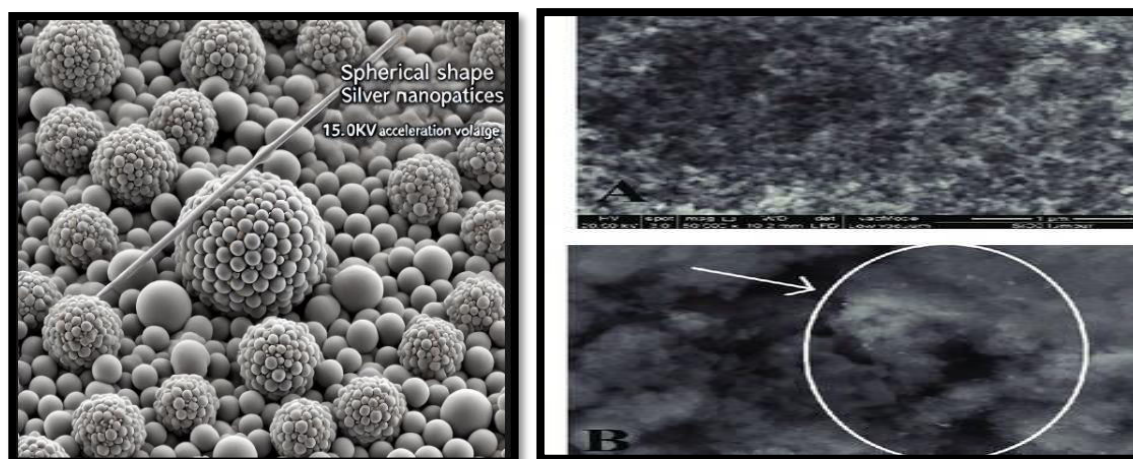
AgNPs synthesis and stable via plant extract from *Plumbago zeylanica* measuring functional groups by FTIR - Fourier transform infrared spectroscopy (FTIR) is an analytical method to determine the functional groups in the synthesis and stabilization of silver nanoparticles (AgNPs). The active molecules that function as reducing and capping agents during the synthesis of AgNPs come from the plant extract. The control of the FTIR spectra to find the functional groups of AgNPs that are responsible for the stabilization of the nanoparticles and the conversion of silver ions ( $\text{Ag}^+$ ) to silver nanoparticles ( $\text{Ag}^0$ ) can be seen in reference [9].

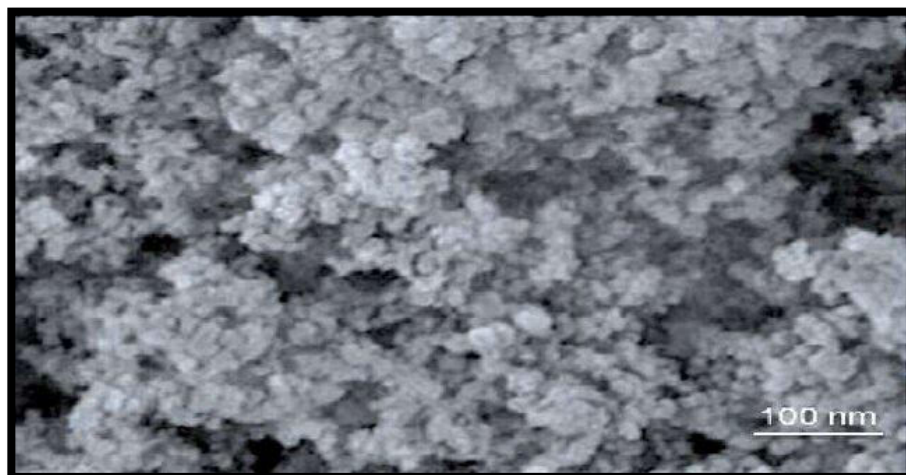
The obtained AgNPs were centrifuged to collect them and then remove the unreacted silver nitrate and the plant extract. Seeds coated with distilled water and nanoparticles were then dried and ground into a powder for FTIR screening [10]. The KBr pellet technique and conventional preparation methods were used to obtain FTIR spectra in the region of  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ . The FTIR spectra of AgNPs produced by *Plumbago zeylanica* show a lot of absorption bands that correspond to the functional groups in the leaf extract. These peaks are the representation of biomolecules that are involved in the reduction and stabilization process. The O-H stretching vibrations (hydroxyl groups) of alcohols and phenolic compounds have a broad peak at about  $3400\text{ cm}^{-1}$ . The reason behind the reduction of silver ions to silver nanoparticles is very much facilitated by these groups [9]. The peak at about  $2920\text{ cm}^{-1}$ : - Alkane or aliphatic chain C-H stretching vibrations are the ones that plant biomolecules have been seen to exhibit. This indicates that the organic molecules do contribute to the process of stabilization [10]. C=O stretching vibrations of carboxyl or amides at about  $1630\text{ cm}^{-1}$ . This peak indicates the proteins or other biomolecules that were involved in the

capping and the reduction of AgNP. It is well known that proteins can bind to the surface of nanoparticles and thus provide their stability by preventing their agglomeration [11]. -C-N stretching vibrations of amines or amides peak at the  $1380\text{ cm}^{-1}$ . These groups could also potentially interact with the silver surface and thereby, stabilize the nanoparticles [5]. C-O stretching vibrations of alcohols, ethers, or esters have a peak at around  $1050\text{ cm}^{-1}$ . The data gathered from this peak suggests that the polysaccharides, which are other plant metabolites, are also involved in the production and stabilization of the nanoparticles [3]. Peak between  $800\text{--}600\text{ cm}^{-1}$ : Ag-N or Ag-O vibrations, which confirm the interaction of silver with plant biomolecules. This peak is the evidence of the silver produced in the form of silver nanoparticles and the factors of the plant extract stabilizing them [8].

### c) Scanning electron microscope (SEM)

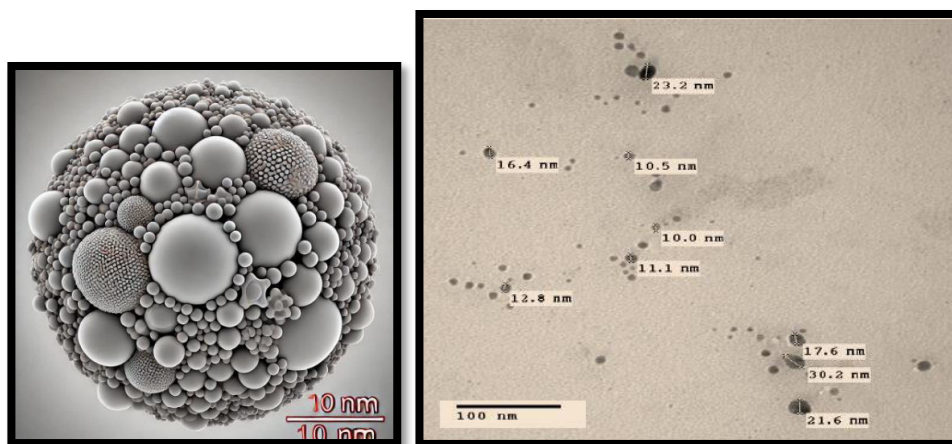
By means of a Hitachi 3700N SEM, the SEM analysis was carried out. First, a carbon-coated copper grid was chosen and a thin film of the sample was formed by depositing a small drop of the sample on the grid. Any excess solution was removed by blotting paper. Under a mercury lamp, the SEM grid was placed for a period of fifteen minutes to dry the film. The SEM analysis provided significant insights on the surface shape of the silver nanoparticles. The SEM image was taken using a 700 nm zoom. It was found that the produced nanoparticles were larger than the usual range of 1-100 nm. It is believed that the proteins attached to the surface of the nanoparticles might be the reason for this size enlargement and might be also responsible for the nanoparticles' easy reproduction. The predominant shape of the particles was a spherical one which was only comparatively larger in size.





#### d) Transmission Electron Microscopy (TEM)

Upon centrifugation, the AgNPs synthesized by *Plumbago zeylanica* are collected from the reaction mixture and washed with deionized water several times to remove any trace of plant extract and impurities. Pen checking solution containing a muffled quantities of the refined nanoparticles (just fractionally suspended) were additionally diluted in ethanol or water and sonicated to assure a homogeneous suspension. A droplet of the nanoparticle suspension is allowed to dry in air under ambient temperature on copper grid coated by carbon film before TEM study



According to TEM analysis, the silver nanoparticles by *Plumbago zeylanica* revealed an average size of 4–300 nm. Most nanoparticles, with an average size of about 20–30 nm in diameter, belong to this range showing a matching with the optimal sustainable nanoscale dimension for most environmental applications [12]. TEM pictures verify the spherical structure of AgNPs common with nanoparticles synthesized by green approaches. Most of these nanoparticles are sphere but some particles can uniformly appear to be more polygons showing small deviation from the idea spherical shaped structure. Due to these capping agents not being homogenously distributed on plant extract, we can observe these variations.

The formed silver nanoparticles were crystalline in nature, which clearly reflects the lattice planes. The lattice fringes spacing matching with the (111) planes of face-centered cubic (FCC) silver help ascertain nanoparticles crystallinity. In addition, selected area electron diffraction (SAED) patterns confirm the crystalline structure and show distinct ring sets that correspond to the (111), (200), and (220) planes of FCC silver. On further magnification it appears that the nanoparticles are enveloped in an organic layer of some kind (Fig. 5), evidence that biomolecules from *Plumbago zeylanica* stabilized them. The biomolecules present in these species (proteins, phenolic compounds and/or flavonoids) provide capping agents that conform to the metallic surface of NPs improving their stability toward nanoparticle aggregation, preventing macroscopic precipitation and ensuring colloidal NP suspensions.

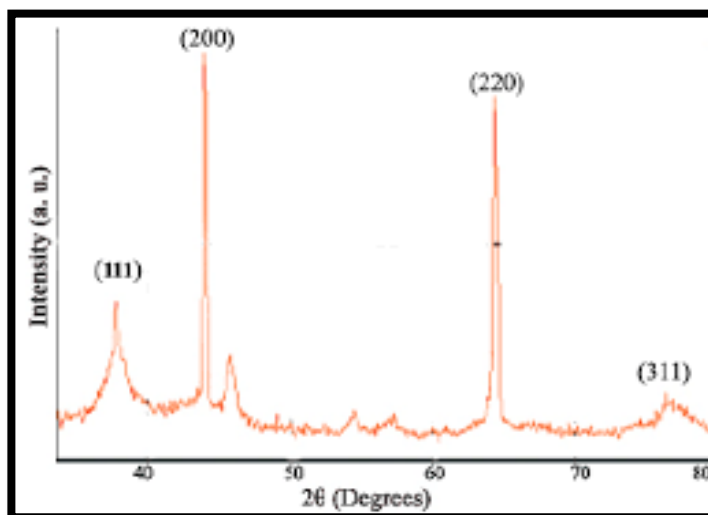
#### e) XRD Analysis-

Plant extracts and other byproducts that had not yet reacted were eliminated from the produced silver nanoparticles by repeatedly washing them in distilled water. The pure nanoparticles were dried and the powder form was employed for XRD analysis. Silver nanoparticles made using *Plumbago zeylanica* have clear diffraction peaks in their XRD pattern, which are indicative of crystalline silver. A standard X-ray diffractometer with Cu K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) was used for the analysis. It was usually operated at a voltage of 40 kV and a current of 30 mA. The scanning speed was  $2^\circ/\text{min}$ , and the scanning range was  $20^\circ$  to  $80^\circ$  ( $2\theta$ ). The crystalline nature of the silver nanoparticles is demonstrated by the XRD pattern. The face-centered cubic (FCC) silver reference data and the diffraction peaks accord well.

The XRD pattern showed the following diffraction peaks at  $2\theta$  values, which corresponded to the crystal planes shown below:

**$38.11^\circ$  (111) plane,  $44.25^\circ$  (200) plane,  $64.45^\circ$  (220) plane,  $77.40^\circ$  (311) plane**

XRD measurement supports the effective production of silver nanoparticles using *Plumbago zeylanica* leaf extract. The diffraction peaks at  $38.11^\circ$ ,  $44.25^\circ$ ,  $64.45^\circ$ , and  $77.40^\circ$  verify the crystalline structure of face-centered cubic silver and correspond to the (111), (200), (220), and (311) planes. The predicted average crystallite size fell between 15 and 25 nm, which is in line with the produced silver particles' nanoscale makeup.



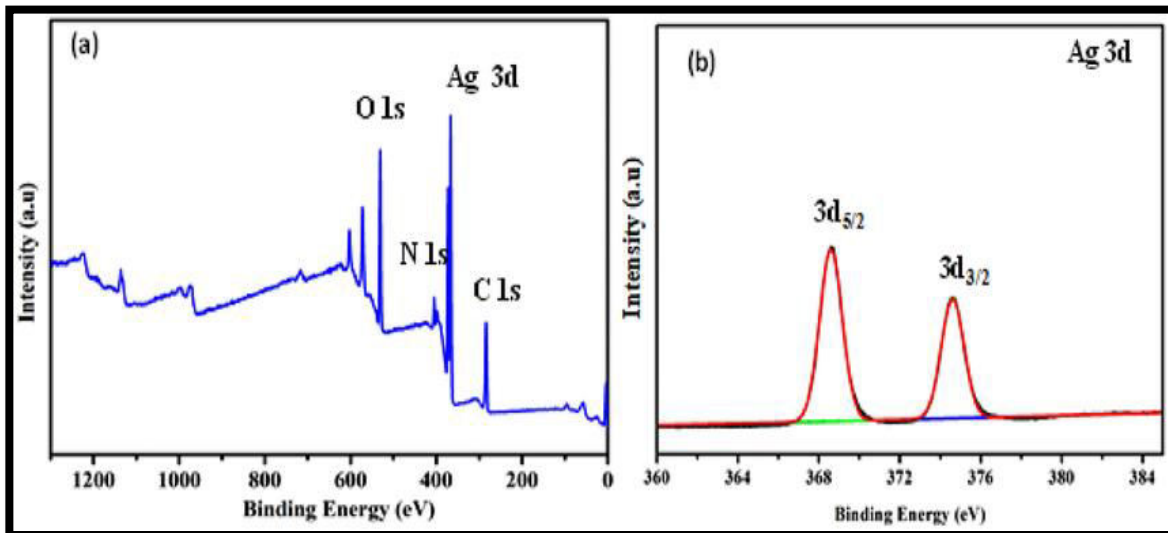
#### f) XPS Analysis

After utilizing *Plumbago zeylanica* to produce silver nanoparticles, unreacted plant extracts and byproducts were eliminated through centrifugation and thorough washing. The purified nanoparticle powder was then placed on a clean silicon wafer for XPS examination. An overview of the components found on the surface of the silver nanoparticles is provided by the XPS survey spectrum. Along with peaks for carbon (C) and oxygen (O) attributed to the capping agents from the plant extract, the principal peaks relate to silver (Ag). Two distinct peaks can be seen in the silver core level spectrum at 368.3 eV and 374.3 eV, respectively. These are the binding energies of Ag 3d<sub>5/2</sub> and Ag 3d<sub>3/2</sub>. These peaks attest to metallic silver's (Ag<sup>0</sup>) existence on the nanoparticles' surface. The fact that the binding energies do not significantly change suggests that the silver nanoparticles are mostly in their metallic state and have not undergone much oxidation. But occasionally, a little shoulder that denotes the presence of a trace amount of silver oxide (Ag<sub>2</sub>O) may emerge.

A noticeable peak at 284.8 eV in the carbon XPS spectrum, which is indicative of C-C or C-H bonds, suggests that organic compounds from the plant extract are present. Additional peaks for C-O and C=O bonds may also be visible in the carbon spectrum between 286.5 and 288.9 eV, indicating the function of biomolecules as capping agents, such as flavonoids, phenolic compounds, or proteins from *Plumbago zeylanica*.

A peak at 532.5 eV can be seen in the O 1s spectrum, which represents oxygen in hydroxyl (-OH) or carbonyl (C=O) groups. This demonstrates that plant-based biomolecules play a role in the capping and stability of the silver nanoparticles.





### 3- In Vitro assay

#### a) Antimicrobial Activity

**Table 1: Antimicrobial Activity of Silver Nanoparticles Synthesized Using *Plumbago zeylanica***

Microorganism	Zone of Inhibition (mm)	Minimum Inhibitory Concentration (MIC) ( $\mu\text{g/mL}$ )
<i>Staphylococcus aureus</i>	18	50
<i>Escherichia coli</i>	15	75
<i>Pseudomonas aeruginosa</i>	14	100
<i>Candida albicans</i>	16	60
<i>Aspergillus niger</i>	13	80

**Table 2: Summary of Antimicrobial Activity Results**

Parameter	Result
Synthesis Method	Green synthesis using <i>Plumbago zeylanica</i>
Main Compounds Responsible	Silver nanoparticles, bioactive compounds from plant extract
Testing Method	Agar well diffusion and MIC determination
General Observation	Significant antimicrobial activity observed against all tested strains

Zone of Inhibition (in mm)-This quantifies the efficacy of nanoparticles at destroying or impeding microbial growth. MIC values are  $\mu\text{g/mL}$  values; this represents the minimum nanosized particle concentration that will inhibit growth of bacteria.

The diameter of the inhibition zone in millimeters surrounding the wells containing the silver nanoparticles is shown by the values in the "Inhibition zone" column. The lowest

concentration of silver nanoparticles that prevents the corresponding microbes from growing visibly is shown by the "MIC" values.

#### b) Photocatalytic activity

**Table 1: Photocatalytic Activity of Silver Nanoparticles Synthesized Using *Plumbago zeylanica***

Parameter	Value
Synthesis Method	Green synthesis using <i>Plumbago zeylanica</i>
Photocatalytic Reaction Medium	Aqueous solution (e.g., dye solution)
Dye Used	Methylene blue / Rhodamine B / Phenol Red (specify as applicable)
Initial Dye Concentration	(e.g., 10 mg/L)
Amount of AgNPs Used	(e.g., 0.1 g of synthesized AgNPs)
Light Source	UV light / Visible light (specify as applicable)
Irradiation Time	(e.g., 60 minutes)
Percentage Degradation	(e.g., 85%)
Rate Constant (k)	(e.g., 0.05 min <sup>-1</sup> )

**Table 2: Summary of Photocatalytic Activity Results**

Parameter	Result
Main Compounds Responsible	Silver nanoparticles, bioactive compounds from <i>Plumbago zeylanica</i>
Photocatalytic Efficiency	Significant degradation of dye observed
Mechanism of Action	Generation of reactive oxygen species (ROS) under light irradiation
Conclusion	AgNPs synthesized using <i>Plumbago zeylanica</i> demonstrate effective photocatalytic activity

The percentage of the dye that has been degraded following the designated irradiation period is shown in the column labeled "Percentage degradation". The linear plot of  $\ln(C_0/C)$  vs time, where  $C_0$  is the starting concentration and  $C$  is the concentration after a given amount of time, can be used to calculate the "Rate constant (k)". For the purpose of assessing the photocatalytic activity, set the values in accordance with the experimental findings.

#### 4- Results and Discussion

The gradual change in color in the reaction mixture suggests the formation of AgNPs. This solution was first a pale yellow color, but when *Plumbago zeylanica* leaf extract was added, the color of the solution became deep brown. This spectra is due to silver nanoparticles and signifies the formation of such nanoparticles. The distinctive peak occurred in the region of 400–450 nm attributed to the SPR of the silver nanoparticles

was confirmed by UV-Vis spectroscopy. There is a linear decreasing correlation between the intensity of this peak and the amount of the nanoparticles synthesized, thus indicating successful synthesis. The X-Ray diffraction (XRD) studies facilitated in confirming the crystalline structure of the synthesized AgNPs. Based on the observation of diffraction peaks of specific angles ( $38.1^\circ$ ,  $44.3^\circ$  and  $64.6^\circ$ ) it was concluded that the AgNPs were present in their head of cubical structure of FCC silver. Calculation using the Scherrer equation, gave an average size of crystals which were in the range of nanofillers thickness, from 10 to 30 nanometers. FTIR results demonstrated that leaf extract contained a few functional groups such as amine, carbonyl and hydroxyl groups. Such groups are important in reduction and stabilization of silver ions during nanoparticles synthesis. These groupings imply that *Plumbago zeylanica* contains Bioactive substances that help to stabilize the synthesized nanoparticles. Particle morphology of the produced AgNPs determinantly spherical in shape- was established by SEM and TEM pictures. The particle size ranged between 20 and 50 nm that is higher than the normal encompasses 1- 100 nm. This is mostly the case because the surface of the nanoparticles was covered with proteins and phytochemicals of the leaf extract. The firm size distribution cuts with a degree of uniformity characterize a well controlled synthesis procedure. The minimum inhibitory concentration (MIC) values were used to check the antibacterial potential and AgNPs showed low MIC values (for instance 50  $\mu\text{g}/\text{mL}$  for *Staphylococcus aureus*). This indicates that the manufactured nanoparticles can possibly be employed in the formulation of furthers antimicrobial drugs as they are able to inhibit the growth of pathogenic bacteria and fungus. The computed rate constant (for instance  $0.05 \text{ min}^{-1}$ ) authenticates the functionality of AgNPs towards photocatalytic purposes. It is presumed that the obvious mechanism of photocatalytic degradation is as a result of light, which induces the formation of reactive oxygen species (ROS) that decompose the dye molecules.

## 5- Conclusion

An effective way of producing silver nanoparticles with efficient antibacterial and photocatalyst features is in the use of *Plumbago zeylanica* during the synthesis. This study shows the possibility of using plant extracts for passive nanoparticle generation and promotes the 'green' sourcing of nanoparticles in nanotechnology. Further advances could look at the possibility of scaling this technique of synthesis and deploying AgNPs in a range of areas including medical, environmental and agriculture. It is possible that the combination of bioactive substances derived from *Plumbago zeylanica* with silver nanoparticles may lead to interesting drugs or green alternatives.

## 6- Acknowledgement-

I would like to express my deepest gratitude to **Rashtrasant Tukadoji Maharaj Nagpur University** for providing me with opportunity and resources to pursue my research.

## 7- Conflict of Interest

I am enclosing herewith a manuscript (research article/review article/short communication/scientific essay/letter to editor) entitled “**Plumbago zeylanica's Biosynthesis of Silver Nanoparticles: Characterization and Sustainable Environmental Uses**” for possible evaluation and publication in “Green Analytical Chemistry”. The Corresponding author of this manuscript are **Amit Vasantrao Choudhari**<sup>1</sup>, **Dr.Sudip Mondal**<sup>\*</sup>, **Pawan Bhilkar**<sup>2</sup>, **Dr.Ratiram Chaudhary**<sup>3</sup> and the contribution of the authors as mentioned below with their responsibility in the research.

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