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Examining Plant-Mediated Green Synthesis of Silver Nanoparticles for Antimicrobial Activity

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ABSTRACT:
In the field of science called nanotechnology, people work on making, manipulating, and using things that are very small. The goal of this study was to protect against harmful bacteria by making silver nanoparticles from Curcuma caesia roxb leaves using eco-friendly ways and then testing how well they work against clinical microorganisms. A characterization of the prepared formulation was done to figure out the shape size and snature of the prepared formulation. One can find out how much the average particle size of the samples changes the shift in SPR wavelengths by measuring the UV-visible optical absorption properties. Additionally, Curcuma caesia leaf extract was used to create silver nanoparticles that were very good at killing harmful bacteria and fungus. The disc diffusion method does a good job of showing the antibacterial action. Antibacterial implantable materials have amazing antibacterial properties that make them good for covering silver-coated devices. They also stop nanoparticles from growing inside the material, which helps wounds heal faster after getting an infection.

Keywords: Nanoparticles, silver, green synthesis, plant based.

INTRODUCTION:

Nanotechnology is the branch of science that studies how to make, change, and use things that are very small, in the nanoscale range. Nanotechnology is coming together with study in herbal and medicinal plant biology. This is becoming clear as technology gets better and scientists learn more (Rajak *et al.,* 2023). One example of this kind of action is using plant sources to make nanoparticles in an eco-friendly way. Silver nanoparticles are easy to make using a variety of methods, including chemical synthesis, which is one of them (Kurian *et al.,* 2016; Aziz and Jassim, 2018; Nataraja et al., 2023; Jaiswal et al., 2023; Komu et al., 2023; Keservani et al., 2024).

Still, most of the chemical processes used to make nanoparticles involve using dangerous and poisonous chemicals that are bad for people's health. For some reason, these chemical processes are also bad for the earth (Singh and Keservani, 2017; Behera et al., 2010; Sharma et al., 2016; Keservani et al., 2019; Sharma et al., 2018; Khulbe et al., 2023; Khairnar et al., 2024; Keservani and Gautam, 2022; Keservani and Gautam, 2020; Keservani et al., 2010; Keservani & Sharma, 2018; Bharti et al., 2012). This shows how much more people want green synthesis and other biological ways to build processes that are good for the world. Sometimes, using different plant materials and their products to make nanoparticles can be more useful than using other biological synthesis methods that need complicated steps to keep microbial cultures alive (Rajamani *et al.,* 2013).

Using plants, fungi, and bacteria to make silver nanoparticles has been studied and written about in a lot of scientific writing. The reducing or antioxidant qualities of these microorganisms usually cause the metal compounds in the nanoparticles they are attached to to break down (Khulbe *et al.,* 2023; Gautam et al., 2015; Khambete et al., 2016; Keservani et al., 2020). Even so, microbe-mediated synthesis is not a useful biological way to make silver nanoparticles for industrial use because it needs to be done in very clean conditions and needs to be maintained. Therefore, plant extracts may be better for this goal than bacteria because they are easier to improve, pose a lower biohazard risk, and need less upkeep for cell cultures (Dubey *et al.,* 2012).

It is a great place to make nanoparticles because it doesn't contain any harmful chemicals and has natural binding agents that keep the nanoparticles stable. Plant-mediated metal nanoparticle synthesis is getting a lot of attention right now because it is easy for anyone to use, makes nanoparticles quickly with a variety of interesting shapes (Ahire *et al.,* 2020), doesn't need a lot of careful cell culture control, and is good for the environment. Plants are the best hosts for biosynthesis because they have important reducing agents like flavonoids, ascorbic acids, citric acid, and external electron shuttlers. It is very important for making metal nanoparticles that these substances are used (Logeswari *et al.,* 2013; Gnanajobitha *et al.,* 2013).

The goal of this study was to find out how well silver nanoparticles made from *Curcuma Caesia Roxb* leaves kill bacteria using a green way. Different types of clinical diseases were used to test the nanoparticles.

MATERIALS AND METHODS:

Extract Preparation:

The Curcuma casia leaves were picked fresh in Hyderabad, India. 20g of finely chopped and thoroughly cleaned leaves were mixed with 100 ml of distilled water in a 500 ml Erlenmeyer flask to make the fresh leaf extract for turning silver ions into silver nanoparticles. After this, the liquid was heated up and left to boil for about five minutes. After that, it was let cool. After filtering it through Whatman filter paper, the extract was kept at a temperature of 4°C for later steps (Sable *et al.,* 2023).

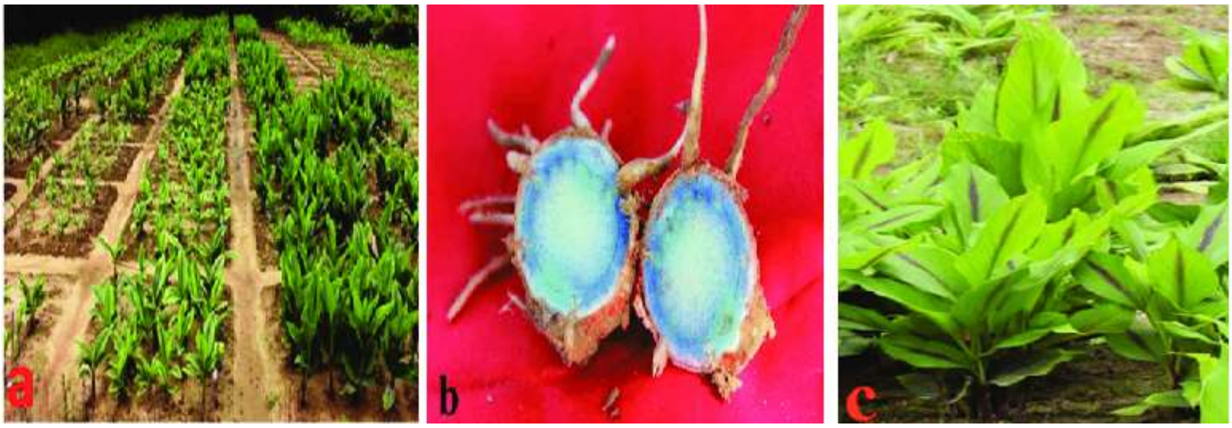

Figure 1: The leaves, stems, and roots of the Curcuma Caesia plant

Formulation of Silver Nanoparticles:

In a 100 cc standard flask, the leaf extract solution is mixed with a 1 mM silver nitrate (AgNO3) solution from Merck, India. The solution is made at room temperature. To lower the amount of Ag+ ions, 10 ml of Curcuma caesia leaf extract was mixed with 90 ml of a water solution that had 1 mM silver nitrate in it (Ahirrao *et al.,* 2023; Netala *et al.,* 2015).

Evaluation of Prepared silver Nanoparticles:

Characterization by using UV-Spectrophotometer:

A UV-VIS spectrophotometer was used to measure the visual properties of silver nanoparticles (AgNPs) made from leaves. Once the AgNPs were formed, measurements were made in the wavelength range of 300–800 nm. Studies done in the past have shown that the absorption in the UV-VIS spectra happened between 390 and 470 nm because tiny silver particles were present. The AgNPs' UV-VIS spectra showed a clear and strong surface plasmon resonance peak at about 419 nm (Behera *et al.,* 2013).

FT-IR Study:

We used an IR-Prestige-21 Shimadzu FT-IR spectrophotometer and the KBr pellet method to get the Fourier transform infrared (FT-IR) spectra of Curcuma Caesia leaf juice and silver nanoparticles. In the 4000-400 cm-1 band, the spectra were taken (Shukla *et al.,* 2017; Ahire *et al.,* 2020).

Morphology study by SEM:

Scientists used a JSM-6390 scanning electron microscope (SEM) to study the silver nanoparticles. A scanning electron microscope was used to look at the anatomy. The scanning electron microscope (SEM) proved the silver nanoparticles' sizes, shapes, and crystal structures (Meenakshi *et al.,* 2017).

Morphology study by TEM:

High-resolution transfer is the process of sending information or data that is very clear and full of details. used a JEM 2100 electron microscope to look at silver nanoparticles. A Transmission Electron Microscopy (TEM) test is a good way to check the qualities of silver nanoparticles that were made using environmentally friendly methods. AgNPs spread out in a liquid. The spread is put on a transmission electron microscopy (TEM) grid, like a copper grid that has been covered with carbon. You can dry the sample either in normal air conditions or by using a dryer (Nataraja *et al.,* 2022).

XRD Analysis:

We studied solid metallic silver nanoparticles with a Bruker D8 Advance X-ray diffractometer that was set to 40 kV/35 mA and used a Cu K± radiation source. In a controlled experiment setting, the X-ray diffraction (XRD) results were collected from 3° to 50° angles (Gupta *et al.,* 2017).

Antimicrobial Activity Study:

A pure culture from the plate was used to seed the Nutrient Agar plate, which was then kept at 37oC for 24 hours. By adding a fresh culture to a clean 2 ml tube with 0.145 mol/L saline solution, a bacterial suspension with a concentration of 1.5×108 colony forming units per milliliter (cfu/ml) was made. After that, the cell density was changed to meet the 0.5 McFarland turbidity standard. A standard inoculum is used in an antibiotic test (Nasr *et al.,* 2019).

RESULTS AND DISCUSSION:

Preparation of Leaf Extract:

To make the fresh leaf extract for turning silver ions into silver nanoparticles, 20 grams of finely chopped and clean leaves were put into a 500-ml Erlenmeyer flask with 100 ml of pure water. After that, the liquid was heated up and left to boil for about five minutes. After that, it was let cool. After being filtered through Whatman No. 1 filter paper, the extract was kept at 4°C for later use in other experiments (Bansal and Kaur, 2020).

Figure 2: ***Extract of Curcuma Caesia*** **Leaf with AgNO3**

Synthesis of Silver Nanoparticles:

A 100 ml standard flask is used to make a 1mM solution of silver nitrate (AgNO3, Merck, India) in water. Then, at room temperature, the solution is mixed with the leaf extract solution. 10 ml of Curcuma Caesia leaf extract was mixed with 90 ml of a water solution (Bansal and Kaur, 2020; Abd *et al.,* 2019).

UV-Visible Spectroscopy:

A UV-vis spectrophotometer, which measures wavelengths between 200 and 800 nm, is used to check that AgNPs are formed and stay stable in pure water. We confirmed that the Ag+ ions were changed to Ago by mixing an extract of Curcuma Caesia leaves with a water-based solution of the silver ion complex and watching the UV–vis spectrum of the reaction medium. As a function of response time, the UV-vis spectra were found. Figure 2 shows how the color of the solution changed over time from yellow to dark brown. The UV-visible spectrum of silver nanoparticles is shown in Figure 2a. The surface plasmon resonance at 446 nm confirms that AgNPs were made.

Fourier-Transform Infrared Spectroscopy:

Fourier Transform Infrared (FT-IR) was used to look at the functional groups in the extract from Curcuma caesia leaves. The results can be seen in Figure 3. The extract of Curcuma caesia has several absorption peaks, which shows that it is complicated. When the N–H bonds in amino groups get longer, they create a peak at 3478 cm −1, which shows that there is a linked hydroxyl (-OH) group there. A strong absorption peak can be found at 2883 cm −1. This is because the functional groups –CH3 and –CH2 are stretching vibrations. The shoulder peak at 1641 cm-1 was given to the carbonyl group (C=O) of amide groups. It's possible that the absorption peaks at 1083 cm −1 are caused by the carboxyl C–O stretching. The main reason why silver ions are turned into silver nanoparticles, according to FTIR study, is that the extract of Curcuma caesia leaves has carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) groups.

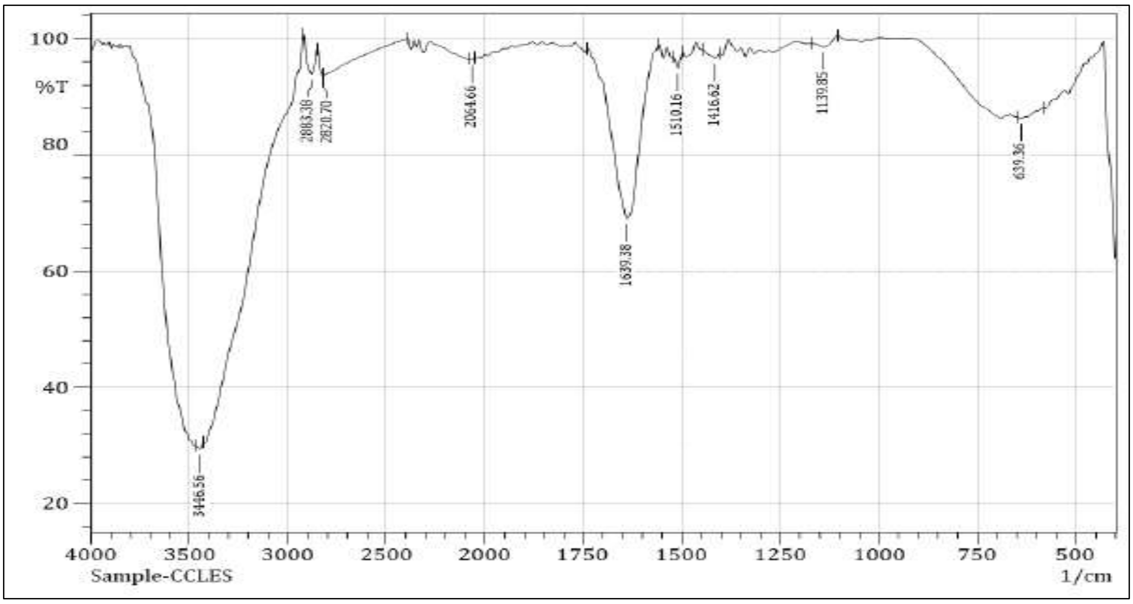

Figure 3: The FTIR spectrum of extract

SEM Study:

A small amount of silver nanoparticles mixed in clean distilled water were put on clean electric sticks so that a scan electron microscopy experiment could be done. After that, the water was left to dry out fully. Figure 4 shows a scanning electron microscopy picture of silver nanoparticles. The nanoparticles had a flake-like structure and a shape that was always the same, with a width of 200 to 350 nm. Based on what the scanning electron microscope (SEM) showed, the smaller silver particles may have joined together to make the larger ones. EDAX gave the details about the atomic proportion and the elements that made up the substance (Mogole *et al.,* 2021; Bangale and Ghotekar, 2019).

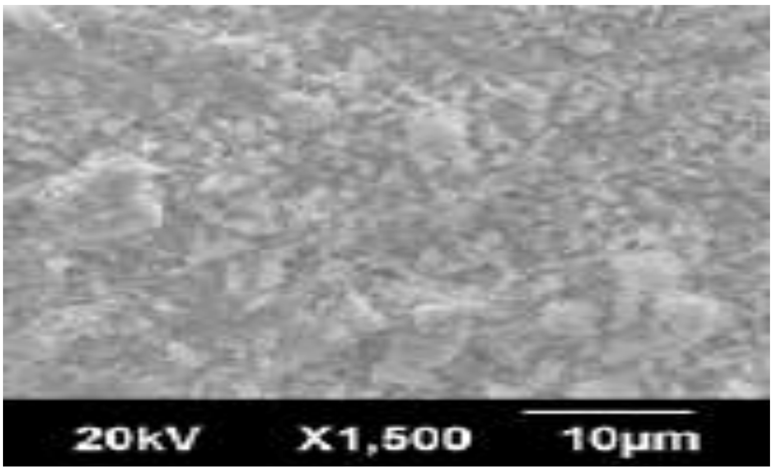

Figure 4: SEM of silver nanoparticles

TEM Analsysis:
Images from transmission electron microscopes are very clear in terms of space. The TEM results show that the nanoparticles that were made are all the same size and are at the nanoscale. The information in Figure 5 shows that the nanoparticles that were made are mostly round, with a diameter that runs from 15 to 60 nm. We also used the SAED pattern to see how the nanoparticles were structured in a solid way. The results showed that the material was solid because they showed a ring pattern with bright dots on a dark background (Mogole *et al.,* 2021; Bangale and Ghotekar, 2019).

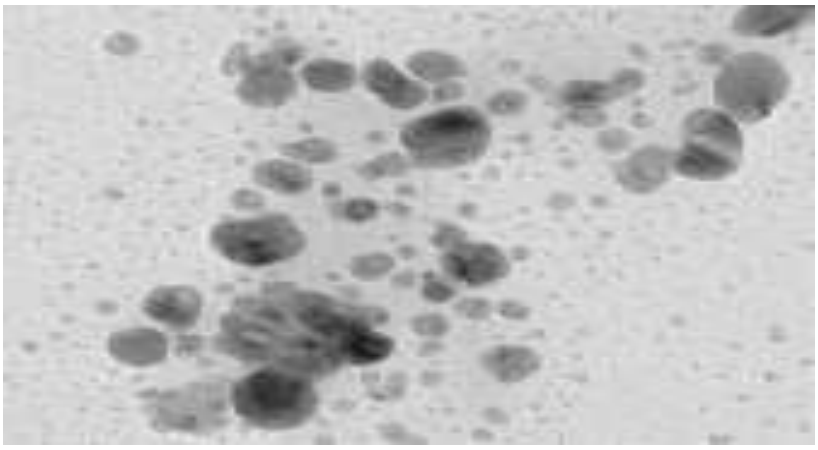

Figure 5: TEM of silver nanoparticles

X-Ray Diffraction Study:
To look at the solid structure of the silver nanoparticles, X-ray diffractograms were used. The XRD pattern showed a lot of Bragg reflections that were easy to spot and match with silver's face-centered cubic structure. By measuring X-ray diffraction, it is easy to see that the silver nanoparticles made when the Curcuma caesia leaf extract reduces silver ions are solid. It was found that the average size was about 16 nm by using the Debye-Scherrer equation and looking at XRD data. The appearance of structural peaks in the XRD signals and the average crystalline size of about 16 nm make it clear that the AgNPs we made using our eco-friendly method were in the nanocrystalline form. Figure 6 shows the average particle size of silver nanoparticles made with this method, which is safe for the environment (Mogole *et al.,* 2021; Bangale and Ghotekar, 2019).

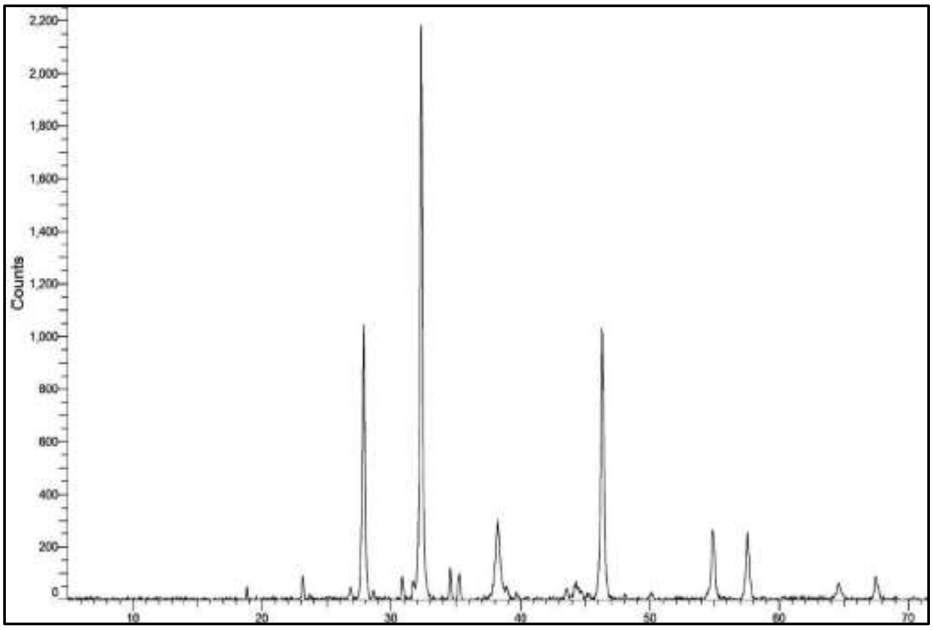

Figure 6: XRD of synthesized nanoparticles

Antimicrobial Assay:

To make the medium, 38 grams of Muller Hinton Agar Medium were mixed with 1000 milliliters of pure water. The solution was made germ-free by putting it in an autoclave for 15 minutes at 121°C and 15 pounds of pressure. The medium that had been in an autoclave was put on Petri plates after it had cooled down and been mixed fully. After that, harmful bacteria like Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, and E. coli were swabbed on the plates. Finally, the plates were put in an incubator that was set to 37°C and left there for 24 hours (Mogole *et al.*, 2021; Bangale and Ghotekar, 2019). It was put on the Mullar-Hinton medium and about 10 µL of the material was added to it before it was given time to grow. The antibiotic study that was done on several bacteria is shown in Table 1.

Table 1: Anti-bacterial study

Sr. No.	Bacterial Used	Positive control	Inhibition Zone
1	E.coli	22	11
2	Klebsiella pneumoniae	24	20
3	Staphylcoccus epidermidis	22	16
4	Staphylococcus aureus	24	12

CONCLUSION:

We present the first results of our research into making silver nanoparticles using a green chemistry method, specifically by using Curcuma caesia leaf extract in a water-based solution that is kept at room temperature. By measuring the UV-visible light absorption properties, one can find out how much the average particle size of the samples changes the bands where surface plasmon resonance happens. Additionally, a leaf extract of Curcuma caesia was used to create silver nanoparticles that were very good at killing harmful bacteria and fungus. The disc diffusion method does a good job of showing the antibacterial action. The nanoparticles that are made can be used to kill germs and clean water. In the nanotechnology processing fields, this method is an option to making a lot of silver nanoparticles that is both cheaper and better for the environment.

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None
Conflict of Interest
None

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