10. Green Synthesis of Silver Nanoparticles with Nycanthes Arbor-Tritis Leaf Extract

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Abstract

Growing concern over environmental pollution and the need for sustainable practices have led to the search for green synthesis methods for nanoparticles. This project focuses on the green synthesis of silver nanoparticles (AgNPs) using the leaf extract of *Nyctanthes arbor-tristis*, commonly known as night-flowering jasmine. The objective of this study is to develop a simple, cost-effective and environmentally friendly method for the synthesis of AgNPs by replacing conventional chemical-based synthesis approaches. The project involved collection of fresh leaves from *N. arbor-tristis*, followed by formation of aqueous leaf extract. The extract was then reacted with silver nitrate for the synthesis of AgNPs. Various characterization techniques such as Colour change detection, UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) were used to analyze the synthesized nanoparticles. Preliminary results indicate the successful synthesis of spherical AgNPs with an average size range of 10.21 nm. UV-Visible spectroscopy exhibited a characteristic surface Plasmon resonance peak around 440 nm, confirming the formation of AgNPs. FTIR analysis revealed the presence of bioactive compounds. The crystallinity of the synthesized Silver nanoparticles was confirmed using an X-ray diffractometer. Furthermore, the antibacterial activity of the synthesized AgNPs was evaluated against selected pathogenic strains, which showed significant inhibitory effects. Overall, this project highlights the potential of *N. arbor-tristis* leaf extract as a green and sustainable source for the synthesis of AgNPs. The obtained nanoparticles have promising antimicrobial activity, which makes them suitable for various biomedical and environmental applications. The use of plant extracts for nanoparticle synthesis provides an environmental friendly alternative to conventional chemical methods, contributing to the development of green nanotechnology.

Keywords - Green synthesis, silver nanoparticles, *Nyctanthes arbor-tristis*, leaf extract.

Introduction

Medicinal plants have been used as a major source of drugs for centuries. Indian culture has a rich history of knowledge on plant-based medicines for use in preventive and curative medicine. Out of the global population, most people rely on plant-derived drugs or medicines to fight health problems. Recently, we have seen an increased need for new and effective antimicrobial compounds from natural resources. This is because some drugs found in traditional therapies are becoming less effective against various diseases. Moreover, plant derived medicines have many benefits that make them more acceptable than synthetic drugs. We should aim to derive the maximum benefit from medicinal plants while still providing adequate health services to those in rural areas. This will require combining modern scientific advances with traditional wisdom (Devi et al., 2020).

Nanotechnology has emerged as a promising escalating field with plethora of applications in numerous areas including medicine, pharmacology, sensing devices, micro-electronics and drug delivery etc. There are many types of nanoparticles, with benefits and drawbacks depending on the type. Organic nanoparticles (carbon, fullerenes), inorganic (magnetic and noble metal) nanoparticles, and semiconductor nanoparticles (titanium oxide and zinc oxide) can all be synthesized using different methods. Inorganic metal nanoparticles (gold and silver) are especially interesting because they have many properties that make them better than available chemical imaging drug agents, such as size and functionality. There is growing interest in using plant extracts to create green Silver nanoparticles. Some plant extracts act as reducing agents or stabilizing agents, making synthesis much simpler and cheaper than traditional methods (Devi et al., 2020).

Ayurveda is the ancient Indian medical science based on herbs and herbo-mineral preparations. In Ayurveda, seven metals used therapeutically are gold (Au), silver (Ag), copper (Cu), iron (Fe), lead (Pb), tin (Sn) and zinc (Zn). These are passed through many processes and finally transformed into therapeutic form. Bhasma is the metal based medicine prepared from metals after many systematic processes to raw metal into therapeutic form. Swarna bhasma gold ash is a therapeutic form of gold metal of nanosized particles when evaluated through various tools and techniques. The size of particle was found to be about 56 nm. Swarna bhasma was also analyzed qualitatively and found that the final product is almost pure gold Au. Bhasma is a metallo-medicine in powder form of nano to submicron size. The raw metal is converted into therapeutic form through classical process by repeated incineration and grinding with some herbal juices and other specified matters. Specialty of preparation process is that the whole process is not a chemical based, rather it is fully a mechanical process and chemical properties much differ to nanoparticles prepared through chemical process (Pandey & Pandey, 2014).

Multidrug-resistant organisms MDROs are becoming a growing public health crisis and make many healthcare-associated infections difficult to treat with current antibiotics. Globally, infections caused by MDROs are emerging causes of morbidity and mortality. The development of new antibiotics requires tremendous economic and labor investment and is time-consuming. For these MDRO infections, high doses of antibiotics will be administered and may generate intolerable toxic and adverse effects, which will prompt the development of alternative strategies. The application of nanoparticles provides a potential strategy to manage infections caused by MDROs (Lee et al., 2019).

N. arbor-tristis, also known as the Night-flowering jasmine, Harsinghar, or Parijata Parvati chi phula, is a species of Nyctanthes native to South Africa and Southeast Asia. It is an important member of Ayurveda, the traditional Indian medicine science. The generic name Nyctanthes came from Greek word Nyktha meaning night and Anthos meaning flower while the specific name arbor-tristis meaning the sad tree because of the dull look of the tree during the day time. *N arbor-tristis* is a small divine ornamental tree, is used to pray God across India and is known for its fragrant white flowers. The plant is a well-known traditional Indian medicinal plant also, which in Ayurveda is used for various pharmacological actions such as anti-arthritic, antispasmodic, antibacterial, anti-inflammatory, immunostimulant, antidiabetic, hepatoprotective, antioxidant, antimicrobial, anthelminthic, antileishmanial, anti-pyretic, anti-allergic, antiviral and CNS depressant. It is an herbal remedy for treating sciatica, malaria, enlargement of spleen and other various infectious and non-infectious diseases. It is also used as blood purifier (Rawat et al., 2021).

N. arbor-tristis is a medicinally important plant of family Oleaceae and exhibits pharmacological activities like antioxidant, and antimicrobial activities. Some bioactive compounds like flavonoids, terpenoids, phenolic compounds, tannin, saponin and reducing sugars are present in the leaves of this plant which has antimicrobial activity. These properties of plant can be enhanced by production of Silver nanoparticle from the extract of different parts of this plant. There is no report of photocatalyzed green silver nanoparticle synthesis using ethanol leaf extract of *N. arbor-tristis* (Mishra et al., 2019).

The objective of the present study was to optimize the different factors for efficient green synthesis of silver nanoparticles and to characterize it by using different techniques. These nanoparticles were tested against different human pathogenic bacteria to confirm the antibacterial activity of green synthesized nanoparticles for its application as an anti-bacterial agent.

Materials and Methods

Preparation of Leaf Extract

N. arbor-tristis leaves were collected from local areas of Akola district, Maharashtra. Leaves were sundried for 10-15 days. Then fine powder of leaves was made using grinder. 25gm of the powder made from crushed leaves was taken. 100 ml of distilled water were combined with the 25gm of powder. Transferred the mixture into a conical flask and rotated it for four hours to ensure proper mixing. Whatman's Filter Paper No. 1 was used to filter the extract.

Synthesis of Silver Nanoparticles

To create the solution mixture, the leaf extract and $AgNO_3$ (0.01 M) were combined in an equal volume. The reaction was then left out all night to finish. After the color change indicated the conversion of silver nitrate ($AgNO_3$) into silver nanoparticles, the solution was centrifuged at 3,000 rpm for 20 minutes. The silver pellet was collected afterwards and further washed 3 times using 5 ml deionized water. It was then centrifuged for 15 minutes to remove impurities. Finally, it was dried overnight in room temperature.

Antibacterial Assay

Sterile nutrient agar plates were prepared. The uniform lawn of cultured pathogenic bacteria (*E. coli, S. aureus, K. pneumoniea, P. vulgaris and P. aeruginosa*) was prepared by swabbing on sterile nutrient agar plates. Using well borer, well of 8 mm were made on all the petri plates. Using micropipette different concentration of prepared leaf extract and synthesized silver nanoparticles were added in the wells. The concentration kept 25µl, 50µl, 75µl, 100µl respectively. The plate was then kept for incubation at 37°C for 24 hrs in an incubator. The zone of inhibition around the wells was observed and measured using zone reader scale.

Characterization

AgNPs were characterized by UV-Visible spectroscopy, X-ray diffractometry and Fourier Transform Infrared spectroscopy. The powder XRD technique was performed for phase identification of AgNPs. FTIR was performed to check the functional groups that was present in the *N. arbor-tristis* extract an on the surface of AgNPs. The effect of volume of leaf extract and $AgNO_3$ on the amount of nanoparticles synthesis was performed.

Result and Discussion

Addition of AgNO3 solution to leaf extract of *N. arbor-tristis* resulted in color change from reddish brown to brownish black within 24 hours. Change in color was observed owing to excitation of 'Surface Plasmon Resonance' highest peak was observed at 440 nm in UV-visible spectrophotometer.

Determination of Antibacterial activity

The antimicrobial activity of silver nanoparticles was observed. *N. arbor-tritis* being a medicinal herb also possesses antimicrobial activity. This antimicrobial activity of both silver nanoparticle and leaf extract was observed against five different pathogenic organisms (*S. aureus, P. vulgaris, K. pneumonia, E. coli and P. aeruginosa*) by clear zone of inhibition. Different size of zone of inhibition were obtained around well of different concentration.

Leaf extract of *N. arbor-tritis* showed maximum zone of inhibition against *K. pneumoniae* (16mm), while it did not show zone of inhibition against *P. vulgaris* and *S. aureus*. It showed minimum zone of inhibition against *E. coli* and *P. aeruginosa* of 13mm.

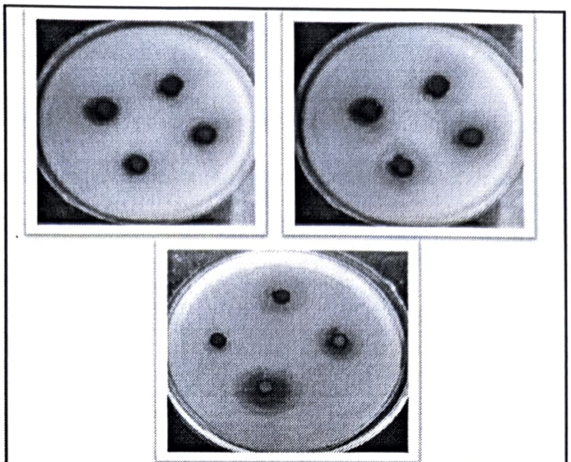
Silver nanoparticles of *N. arbor-tritis* showed maximum zone of inhibition against *K. pneumoniae* (26mm) and minimum zone of inhibition against *S. aureus* (16mm). While it showed no zone of inhibition against *P. vulgaris*. After incubation results indicated that the zone of inhibition of silver nanoparticle is more than that of leaf extract.

Table 1 shows effect of *N. arbortristis* leaf extract on pathogenic bacteria

Sr. No.	Bacteria	Zone of inhibition (in mm) in different concentration of leaf extract			
		25 ul	50 ul	75 ul	100 ul
1.	*S. aureus*	-	-	-	-
2.	*K. pneumonia*	10	12	14	16
3.	*E. coli*	-	10	11	13
4.	*P. vulgaris*	-	-	-	-
5.	*P. aeruginosa*	10	12	13	13

Table 2 shows effect of Silver nanoparticle on pathogenic bacteria

Sr. No.	Bacteria	Zone of inhibition (in mm) in different concentration of silver nanoparticle			
		25 ul	50 ul	75 ul	100 ul
1.	*S. aureus*	-	-	-	16
2.	*K. pneumonia*	13	14	19	26
3.	*E. coli*	13	15	16	21
4.	*P. vulgaris*	-	-	-	-
5.	*P. aeruginosa*	15	17	19	19



Photoplate 4: Zone of inhibition shown by silver nanoparticle

Effect of volume of Leaf extract and $AgNO_3$ on concentration of silver nanoparticles

It was found that increasing the concentration of leaf extract increased the concentration of silver nanoparticles. Leaf extract reduces Ag^+ ions, which in turn increases the concentration of silver nanoparticles. The more volume of $AgNO_3$ is used, the faster silver nanoparticles are synthesized.

The maximum Optical density was observed in equal volume of leaf extract and $AgNO_3$ indicating maximum concentration of silver nanoparticles.

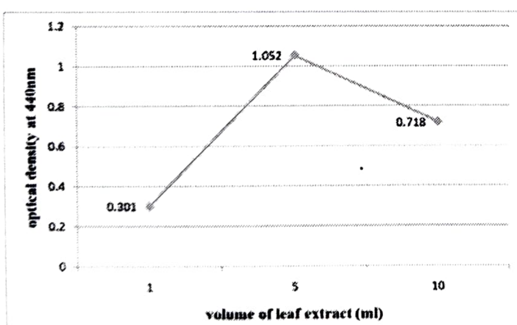


Fig. 1 shows effect of volume of Leaf extract on concentration of silver nanoparticle

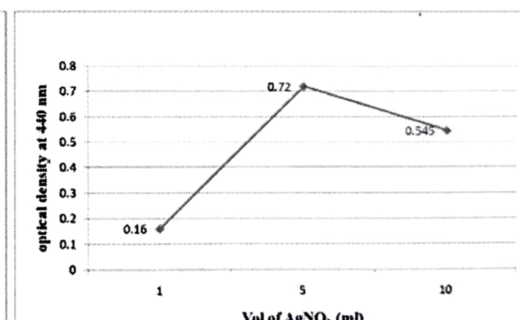


Fig. 2 shows effect of volume of $AgNO_3$ on concentration of silver nanoparticles

X-ray diffraction (XRD) analysis

An X-ray diffractometer was used to confirm the crystallinity of the synthezied Silver nanoparticles. The diffractogram was obtained from the dried layer of the sample over a sample holder.

The average size of the nanoparticles obtained were estimated to be 10.21 nm using Debye-Scherrer Equation,which may indicate a high surface area, and surface area to volume ratio of the nano-crystals.

The equation is written below

$$d = K\,\lambda/\beta \cos\theta$$

Where K, known as Scherer's constant (shape factor), ranges from 0.9 to 1.0, λ is 1.5418Å, which is the wavelength of the X-Ray radiation source, β 1/2 is the width of the XRD peak at half height and θ is the Bragg angle (Kulkarni & Kulkarni, 2014).

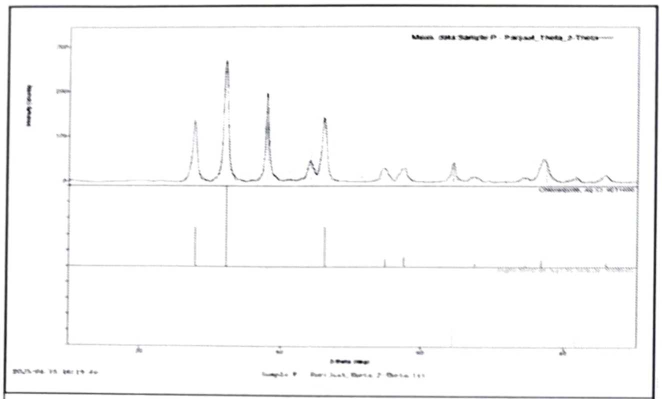


Fig. 3: XRD pattern of $AgNO_3$ nanoparticles

Fourier Transform Infrared (FTIR) Analysis

FTIR helped to detect the functional groups in the extract and the silver nanoparticles that might be accountable for synthesizing the nanoparticles.

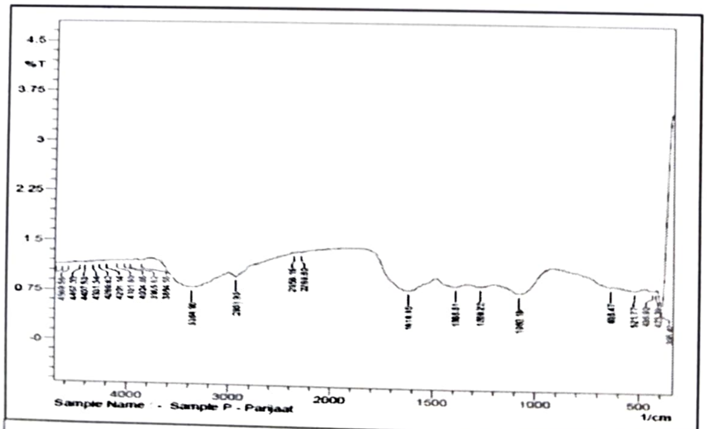


Fig. 4: FTIR pattern of $AgNO_3$ nanoparticles

Conclusion

The present study was an attempt to synthesize silver nanoparticles rapidly by green synthesis method using medicinal leaf extract of *N. arbor-tritis*. It was observed that herbal extract was capable of reducing silver ions and synthesizing silver nanoparticles. Color change reported the primary indication of silver nanoparticle synthesis. Silver nanoparticles possess antimicrobial activity against different pathogenic organisms. Effect of volume of leaf extract and volume of $AgNO_3$ was that increase in these parameters increases concentration of silver nanoparticles. The crystallinity of the synthesized Silver nanoparticles was confirmed using an X-ray diffractometer. FTIR helped to identify the functional groups responsible for creating the nanoparticles.

Acknowledgment

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