

Green Synthesis of Silver Nanoparticles Using *Murraya koenigii* Leaf Extract and Their Antibacterial Potential

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Abstract

The eco-friendly and green synthesis of silver nanoparticles (AgNPs) was done by using the extract of young leaves of medicinally important plant *Murraya koenigii* (Curry Leaves). This method was very simple and rapid multiplication. The green synthesized silver nanoparticles were characterized by FTIR and X-ray diffraction (XRD). To identify the compounds responsible for reduction of silver ions, the functional groups present in plant extract were investigated by FTIR analysis and to observe the crystalline nature, structure was analyzed in XRD. Antibacterial activity of Ag NPs was performed by disc diffusion method against *E. coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Enterococcus faecalis* and *Staphylococcus aureus*. The highest antimicrobial activity of silver nanoparticles synthesized by *Murraya koenigii* extracts was found against *Klebsiella pneumoniae* (20 mm). The result revealed that the plant has potent antibacterial activity and it can be used in drug preparation in future.

Keywords: *Murraya koenigii*, FT-IR, *Pseudomonas aeruginosa*, green synthesis, silver nanoparticle

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INTRODUCTION

Nanotechnology is one of the most active areas of research in modern biological field for the purpose of manufacturing new materials at the nanoscale level [1]. In recent years, various methods have been followed for synthesis of silver nanoparticles. The synthesis of silver nanoparticles was done by physical, chemical and biological methods, but the development of green technology to produce nanoparticles is an important aspect of nanotechnology. Most of photosynthesised plants used in the synthesis of silver nanoparticles in the field of herbal and medicinal plant biology had more attention to nanobiotechnology [2]. Plants have a potent phytochemical in their parts and it is applied in various fields [3]. Biological synthesis of silver nanoparticles provides a wide range of applications; environmentally acceptable methodology, low cost, rapidly growing and eco-friendly. In the recent years, plants, bacteria, viruses, algae and fungi have been used for production of energy-efficient and eco-friendly metallic nanoparticles. Many bacterial pathogens were used for different kinds of nanoparticles; some are gold nanoparticles using *Shewanella* algae; it is a

kind of marine bacterium [4], silver nanoparticles by *Cynobacteria Plectonema boryanum* [5], magnetite nanoparticles by *Actinobactor sp.*, cadmium nanoparticles biosynthesis was done by *Clostridium thermoaceticum* and *Shewanella oneidensis* is used for uranium nanoparticles [6]. Ag NPs were also obtained using *Aloe vera* [7], *Acalypha indica* [8], and *Garcinia mangostana* [9] leaf extracts. Some pathogenic microbes killed by silver nanoparticles are *Bacillus subtilis*, *Klebsiella planticola*, *Pseudomonas sp.* [10, 11], *Vibrio cholerae*, *S. aureus*, *Proteus vulgaris* and *P. aeruginosa* [12], *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Fusarium oxysporum* [13]. *Murraya koenigii* is used in many dishes in India, Sri Lanka and neighbouring countries, which belongs to the family Rutaceae. This work includes green synthesis of silver nanoparticles which were analyzed by Fourier transform infrared spectroscopy and X-ray diffraction assay. Finally, the medical property of the silver nanoparticles were characterized using antibacterial activity against some human pathogens such as *E. coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*,

Enterococcus faecalis and *Staphylococcus aureus*.

MATERIALS AND METHODS

Selection and Collection of Plant Material

The experimental material *Murraya koenigii* (Curry Leaves) was collected from the garden and identified with the help of herbarium specimens deposited in St. Xavier's College (Autonomous) Herbarium (XCH).

Preparation of Leaf Extract

10 g of fresh young leaves of *Murraya koenigii* was thoroughly washed with distilled water for surface cleaning and for removing the unwanted dust particles. Then surface was sterilized with 0.1% HgCl₂ for 1 min to reduce microbial contamination [14]. The sterile leaves were cut into small pieces and boiled with 100 ml of double distilled water for 15 min at 60°C and filtered through Whatman number-1 filter paper. The filtered samples were collected in a conical flask and used for the synthesis of SNPs.

Preparation of Silver Nitrate Solution

1 mM silver nitrate solution was prepared by the concentration of 0.0169 g in 100 ml double-distilled water and stored.

Metal-Plant Extracts Interaction

90 ml of silver nitrate solution was taken in a conical flask. To this, 10 ml of plant extract was added. The color change of the silver nitrate solution was found from colourless to dark brown.

Concentration of Phyto Nanoparticles

After 72 h of incubation, the colour change was observed. This indicated that the SNPs were synthesized from the plant materials with the help of aqueous solution. Then this solution was taken in a centrifuge at 10,000 rpm for 20 min. The pellets were taken after centrifugation and mixed with petroleum ether for rapid drying, dried pellets were collected in a micro-centrifuge tube and the pellets were used for analysis purpose and testing of antimicrobial activity.

FTIR Analysis

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for

10 min and the resulting suspension was re-dispersed in 10 ml sterile distilled water. The centrifuging and re-dispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FT-IR Nicolet Avatar 660 (Nicolet, USA).

XRD Measurement

The characterization of purified synthesized silver nanoparticles were freeze dried powdered and used for XRD analysis (PANalytical X'Pert Pro Powder X'Celerator Diffractometer) at 40 kv/20 mA using continuous scanning 2-delta mode [15]. The silver nanoparticles solution was purified by repeated centrifuges at 5000 rpm for 20 min, followed by re-dispersion of the pellets of silver nanoparticles into 10 ml of deionized water. The deionized water was used as the blank. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern. Powder XRD patterns were recorded using a powder X-ray diffractometer.

Antibacterial Activity of SNPs

The antibacterial activity of isolated plant SNP pellets was tested by agar disc diffusion method. The test organisms used for the assay are *E. coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Staphylococcus aureus* by agar disc diffusion method. Whatman no.1 filter paper of 5 mm diameter was used; these discs were sterilized before use, for the preparation of discs. The extracts of the SNP solution were added to the sterile disc which was incorporated individually with 200–500 µl extract of SNP solution using a micropipette. After incubation at 35°C for 18 h, the different levels of zone of inhibition were observed.

RESULT AND DISCUSSION

Synthesis and Characterization of Silver Nanoparticles

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using curry leaf extracts. Aqueous silver nitrate ions were reduced during exposure to the *M. koenigii* leaf extract. The colour of the reaction mixture changed from yellow to brown which indicates the formation of silver nanoparticle [16]. The

colour change was clear indication for the formation of silver nanoparticles. After reduction for 48 h, culture filtrate colour changed from yellow to brown [17]. This brown colour of silver nanoparticles arises due to the surface plasmon vibrations in the aqueous solution [18].

FT-IR Analysis

FTIR spectrum of *Murraya koenigii* leaf mediated synthesized silver nanoparticles indicated the presence of biomolecules involved in the reduction process (Figure 1). The smaller peak found at 1243.43 cm⁻¹ represents the C-N, P=O stretches are amines

and phosphonate. 1282.64 cm⁻¹ peak may be due to the aromatic and C-O group of amine oxide (N-O) and ester; the peak at 1460.38 cm⁻¹ is due to the C=C stretch of aromatic; the peak at 1635.41 cm⁻¹ shows NH₂, C=O, C=C stretches of amines, amides, alkenes and 2106.97 cm⁻¹ corresponding to Si-H, -N=C=O, -N=C=S -N=C=N-, -N₃, C=C=O, C-C stretches of isocyanates, isothiocyanates, diimides, azides, ketenes, alkyne and silane; 2362.49 cm⁻¹ indicates P-H stretch of phosphine, the major peak at 3303.61 cm⁻¹ is due to the C-H, O-H, N-H stretches of alkyne, alcohol and amines (Table 1).

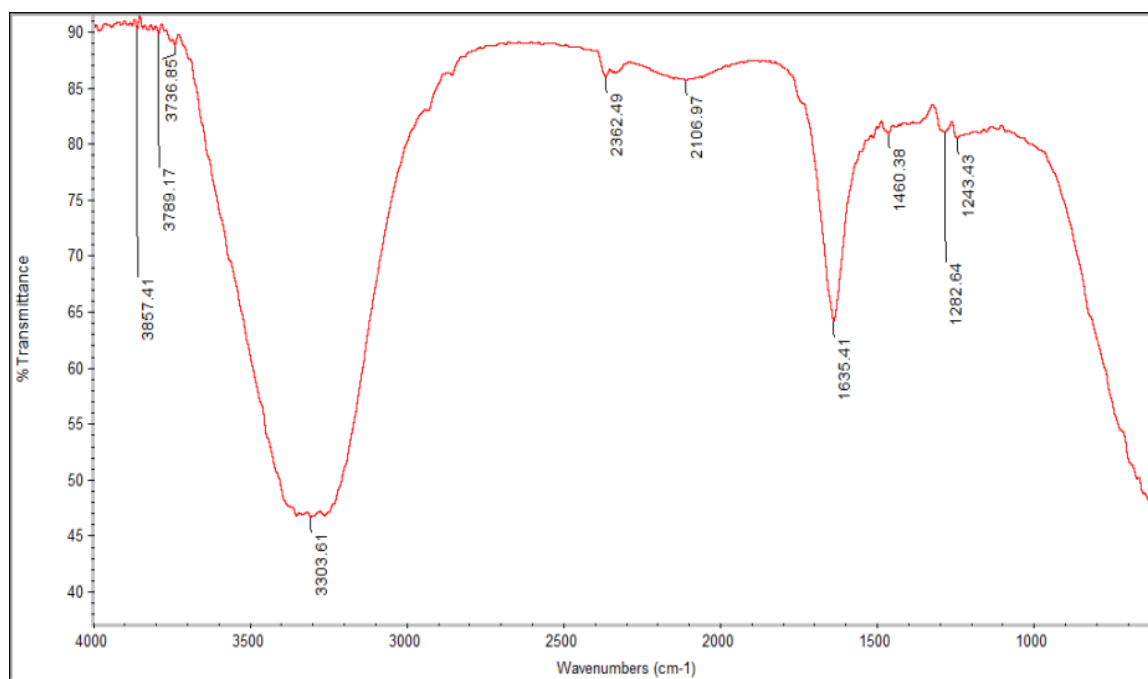


Fig. 1: FT-IR Spectrum of Silver Nanoparticles Synthesized by *Murraya koenigii* (Curry Leaves).

Table 1: FT-IR Spectral Values and Functional Groups *Murraya koenigii* (Curry Leaves).

S. No.	Peak Values	Functional Group	Class
1.	1243.43	C-N, P=O	Amines, Phosphonate
2.	1282.64	Aromatic, C-O	Amine Oxide (N-O), Ester
3.	1460.38	C=C	Aromatic
4.	1635.41	C=C, C=O, NH ₂	Alkene, Amides, Amines
5.	2106.97	Si-H, -N=C=O, -N=C=S-, N=C=N-, -N ₃ , C=C=O, C-C	Silane, Isocyanates, Isothiocyanates, Diimides, Azides, Ketenes, Alkyne
6.	2362.49	P-H	Phosphine
7.	3303.61	C-H, O-H, N-H	Alkyne, Alcohol, Amines
8.	3736.85	Unknown	Unknown
9.	3789.17	Unknown	Unknown
10.	3857.41	Unknown	Unknown

FT-IR revealed that carboxyl and amine groups may be involved in the reduction and stabilizing mechanism. The observed peaks are mainly attributed to flavanoids and terpenoids excessively present in plant extract. FT-IR analysis was studied for synthesized Ag NPs to find out the possible reducing bio-molecules within the *Datura stramonium* leaf extract [19]. Organic functional groups like OH, C=O linked to the surface of nanoparticles are found by FT-IR [20]. A similar result was obtained using gallic acid to synthesize silver nanoparticles with various sizes [21].

XRD Measurement

Structural and crystalline nature of the silver nanoparticles has been performed using XRD analysis. Figure 2 shows the biosynthesized silver nanostructure by using *M. koenigii* leaf extract which was demonstrated and confirmed by the seven characteristic peaks observed in the XRD image at 2θ values ranging from 20° to 80° . A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peak at 2θ value of 38.1842° corresponding to the height of 256.30cts for *M. koenigii*. The corresponding 'd' spacing value of silver nanoparticle is 2.35697\AA for synthesized leaf nanoparticles. A comparison of our XRD spectrum with the standard confirmed that the

silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peak at 2θ value of 38.1842° corresponding to the height of 256.30cts for *M. koenigii*. The corresponding 'd' spacing value of silver nanoparticle is 2.35697\AA for synthesized leaf nanoparticles. The result indicated that the silver nanoparticle synthesized by leaf extract is crystalline in nature. Average size of the particles synthesized was 15 nm with size range 10 to 50 nm with cubic and hexagonal shape. The XRD pattern revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles in papaya fruit extract. The calculated values are concordant with the standard lattice parameter of 0.40729 nm for metallic silver [22].

Antibacterial Activity

In the present study, *Murraya koenigii* leaf extract showed good activity for the zone of inhibition against some human pathogens. The zone of inhibition observed in *Klebsiella pneumoniae* (20 mm) were maximum zone of inhibition against leaf extract synthesized SNPs. The second one *E. coli* was (17 mm) zone of inhibition observed. The *Pseudomonas aeruginosa* and *Enterococcus faecalis* were showed in 16 and 10 mm zone of inhibition. The final one was observed in *Staphylococcus aureus* with very low or absence activity for the zone of inhibition (Figure 3).

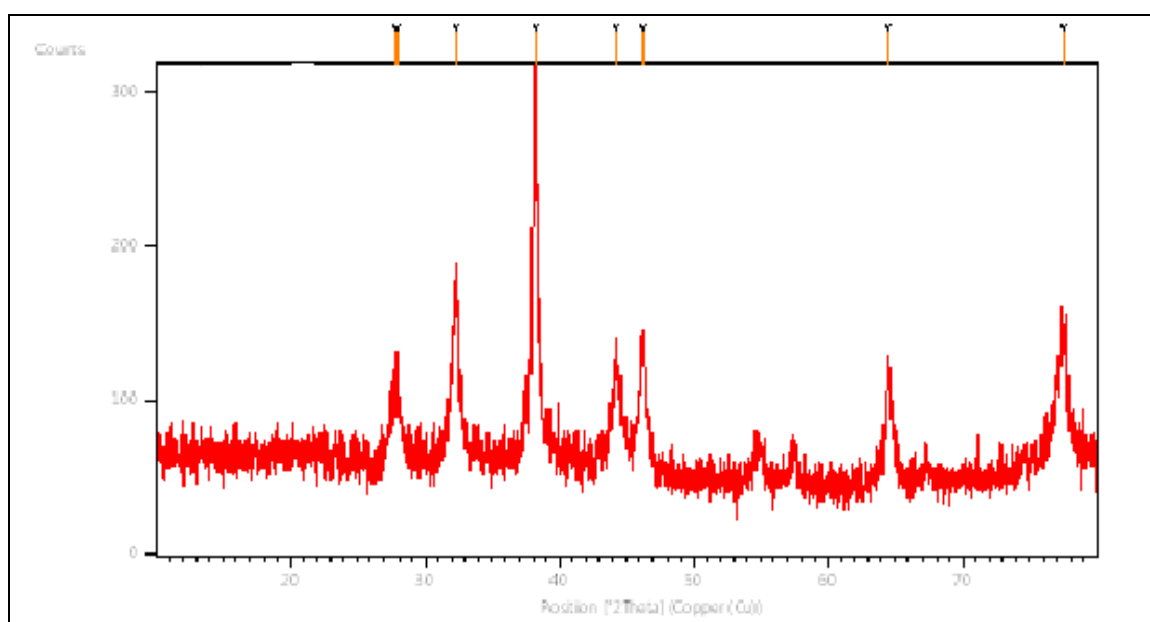


Fig. 2: XRD Analysis of *M. koenigii* Leaf Extract Mediated Synthesized AgNPs.

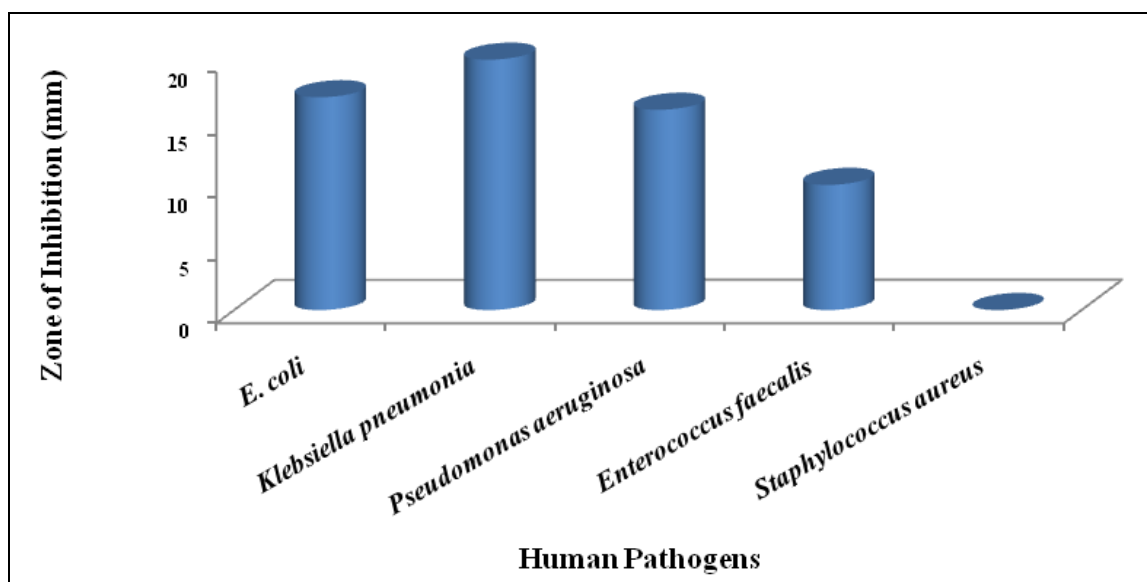


Fig. 3: Antibacterial Activity of SNPs of *Murraya koenigii* Leaf Extract.

It can be provided for the new platform in the field of antibacterial drugs development in biomedical field. The renowned inhibitory effect of silver has been known for many years and used for various medical applications [23]. There are various mechanisms proposed in the literature for the antibacterial activity of silver nanoparticles [24]. The antibacterial activity of silver nanoparticles was due to the breakage of double-stranded DNA molecule in the bacteria [25].

CONCLUSION

The plant-mediated green synthesis of silver nanoparticles was successfully performed and that the leaf extract can also be a good source for synthesis of silver nanoparticles was presented. The sizes of synthesized silver nanoparticles were reported to be 10–20 nm by XRD analysis. FTIR analysis confirmed that the nanoparticles were presented by plant compounds. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, quick and simple, easy to make a different purpose and less toxic etc. The result of antibacterial activity showed that the biological synthesis of silver nanoparticles had a high inhibiting activity against some human pathogens such as *E. coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Enterococcus faecalis* and *Staphylococcus aureus*. So it can be used in many scientific applications like biomedical, drug design and etc.

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