



RAPID GREEN SYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *FICUS AURICULATA*: CHARACTERIZATION AND ANTIMICROBIAL STUDIES.

GURPREET KOUR¹ *, S C SATI², MANISHA SATI³

¹Department of Chemistry, HNB Garhwal University, Srinagar, Pauri Garhwal. Uttarakhand.

²Assistant Professor, Department of Chemistry, HNB Garhwal University, Srinagar, Pauri Garhwal., Uttarakhand

³Lecturer, Department of Chemistry, HNB Garhwal University, Srinagar, Pauri Garhwal. Uttarakhand

ABSTRACT

Ecofriendly and stable silver nanoparticles have been synthesized using aqueous leaf extract of *Ficus auriculata* without using any synthetic reagent. In the present study it was observed that silver ions were reduced by leaf extract within 10 minutes leading to formation of silver nanoparticles. The synthesized silver nanoparticles are characterized by UV-Vis spectroscopy, XRD, High resolution scanning electron microscopy (SEM) and High resolution transmission electron microscopy (TEM). UV-Vis analysis of the sample confirmed the formation of silver nanoparticles exhibiting a peak at a wave length of 437nm. HR-TEM showed that the synthesized silver nanoparticle has spherical shape with a mean size ranging from 23nm to 67nm silver nanoparticles showed antimicrobial activity against *Bacillus cereus* and *Klebsiella pneumoniae*.

KEYWORDS: Timla, Transmission electron microscopy, Green nanochemistry, Antimicrobial activity



GURPREET KOUR *

Department of Chemistry, HNB Garhwal University, Srinagar, Pauri Garhwal. Uttarakhand.

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INTRODUCTION

Nanotechnology is one of the most developing fields of research as it deals with the study of materials having dimension less than 100 nm. Metal nanoparticles have attracted great interest in recent years because of their catalysis, medical and optical properties.¹⁻³ There is a growing need to develop eco-friendly processes to avoid the use of toxic and hazardous chemicals in their synthesis⁴ among all nanoparticles, silver nanoparticles have got significant attention in the fields of agriculture, forensic science, chemistry, waste management, pollution control, solar cells, and biomedicine.⁵⁻⁷ Silver nanoparticles show significant properties depending upon their size, morphology, distribution and also has been recognized as effective antimicrobial agent that exhibits low toxicity in human⁸ Due to potent antimicrobial properties, silver nanoparticles are also utilized in household water filter, clothings, cellphones, cutting boards, kids toys, antibacterial, sprays and detergents, cosmetics etc.^{9,10} A number of methods are used for the synthesis of silver nanoparticle. They are usually synthesized by chemical reduction method, electrochemical method, ultrasonic method, sole-gel method, radiation method and microwave method.¹¹⁻¹⁴ Stabilizer such as polyethylene glycol,¹⁵ Polyvinyl alcohol,¹⁶ polyacrylonitrile¹⁷ are used to prevent nanoparticle agglomeration. Most of these methods involve use of toxic chemicals which may cause environmental risks. Hence the need of hour is to develop ecofriendly processes for nanoparticle synthesis without using toxic chemicals. In comparison to other methods, synthesis of nanoparticles using plant extracts is of low cost, ecofriendly and single step method.¹⁸ Plant extracts are easily available, acts as reducing and capping agents and metalnanoparticles with different size and shape can be comfortably synthesized.¹⁹⁻²¹ *Ficus auriculata* (Timla) is a huge tropical, deciduous and evergreen tree with more than 800 species which belongs to family Moraceae. Preliminary phytochemical screening of the plant leaf extract revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, tannins and reducing sugar. Flavonols contents (kaempferol, quercetin and myricetin) have also been identified.²² *Ficus* species are rich source of polyphenolic compounds, flavonoids which are responsible for strong antioxidant properties

that help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases. Bark, root, leaves, fruit and latex of this plant are frequently used for the treatment of various illnesses. Leaves are crushed and the paste is applied on the wounds. They are also used in diarrhoea and dysentery. The Leaf and fruit parts of *Ficus auriculata* are reported to have antibacterial, anti-inflammatory and antioxidant activities.²³ Although there are number of uses and applications of the plant Timla but little information is available on the synthesis of silver nanoparticles, hence we have commenced this study to synthesize and characterize silver nanoparticles from leaves of timla and also to investigate the antibacterial activities of the synthesized nanoparticles.

MATERIALS AND METHODS

Materials

Timla leaves were collected from the Garhwal region and authentication of the plant was carried out by expert taxonomists of department of Botany in HNB Garhwal University, Srinagar, Pauri- Garhwal, Uttrakhand whereas silver nitrate was procured from Qualigens Fine Chemicals, India. Millipore water was used throughout the study.

Methods

Preparation of Aqueous Extract

The fresh and healthy leaves of *Ficus auriculata* were collected and washed thoroughly with millipore water to remove dust from the leaves.²⁴ The collected leaves were dried in shade. The dried leaves of Timla tree were crushed using a mortar and 10 g of crushed leaves were extracted with 100 ml of millipore water in a clean round bottom flask at 70°C for 60 minutes. After extraction, the supernatant liquid was filtered through a Whatman filter paper. The clean supernatant liquid was used for the synthesis of AgNPs.²⁵

Synthesis of AgNPs

10 ml of the aqueous extract was added to 90 ml of 3 mM AgNO₃ solution in 250 ml conical flask. The total reaction volume was fixed to 100 ml at room temperature. The colour of mixture changed to dark brown which indicated the formation of nanoparticles (Figure 1).

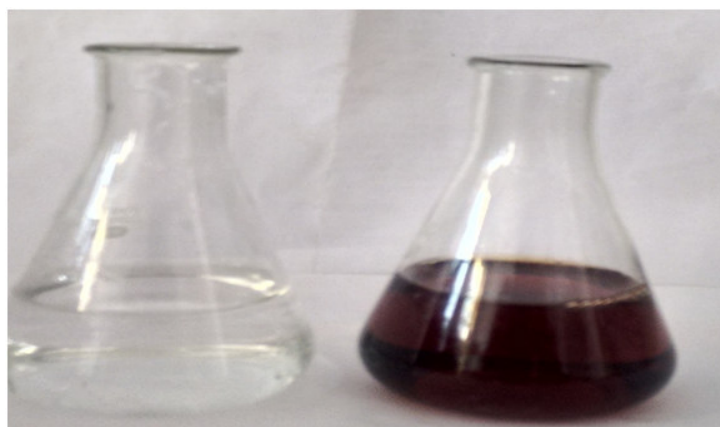


Figure 1

A. Freshly prepared silver nitrate, B. Reaction mixture of leaves extract and silver nitrate

Characterization of Silver Nanoparticles

UV Analysis of Silver Nanoparticles

The UV–Vis spectroscopy analysis was performed with dilution of 0.5 ml of the sample with 2.5 ml of millipore water. The absorbance was calculated over the wavelength range, 200–800 nm (scan rate 2 nm/s). The dual beam spectrophotometer (Perkin Elmer Lambda 25) was used to attain the absorbance vs wavelength curve of silver nanoparticles.

XRD Analysis of Silver Nanoparticles

The X-ray diffraction, using Smartlab (Rigaku), was used to determine the particle size and nature of the AgNPs and it was operated at 40 kV, 40 mA with Cu K α radiation at 2θ angle ranging from 20° to 80° . The crystallite size of the silver nanoparticles was estimated using Debye Sherrer's equation.

$D = 0.94 \lambda / \beta \cos \Theta$, Where D is the average crystallite size, λ is the wavelength of X-ray source (0.15406 nm), β is the full width at half maximum (FWHM), and θ is the diffraction angle.

SEM analysis of Silver Nanoparticles

FESEM (Sigma, Carl Zeiss) instrument was utilized to observe the morphology of the synthesized AgNPs. Thin films of the sample were prepared on a gold coated copper grid by just dropping a very small amount of the sample on the grid. Other particulars concerning applied

voltage, magnification used and size of the contents of the images were implanted on the images itself.

TEM analysis of Silver Nanoparticles

Transmission electron microscopy (TEM) was used to confirm the size shape and surface morphology of the particle. High Resolution Transmission Electron Microscopy (HRTEM) was performed by Tecnai G2 F30 S-Twin (FEI) machine, operated at an accelerated voltage of 300 kV. These images were taken by drop coating AgNPs on a carbon-coated copper grid.

Antibacterial Property of Silver Nanoparticles

The disk diffusion method using a Mueller-Hinton agar culture medium (Hi-media) was used to assess the antibacterial activity of the silver nanoparticles against the human pathogenic bacteria *viz.* *Bacillus cereus* and *Klebsiella pneumoniae*.²⁶ The standard cultures were inoculated 10^8 CFU/ml in petri dishes with MH agar medium and then paper disks of 5mm diameter were laid on the inoculated standard culture, which was instilled with nanoparticles neat solution in DMSO (dimethyl sulfoxide). Petri dishes were incubated at 37°C for 24 hrs and antimicrobial activity was determined by measuring the zone of inhibition around the disk. The inhibition zone that appeared around the disc was measured and recorded as the antibacterial effect of Timla and the various concentrations of nanosilver stabilized in Timla extract.

RESULTS AND DISCUSSION

UV Analysis

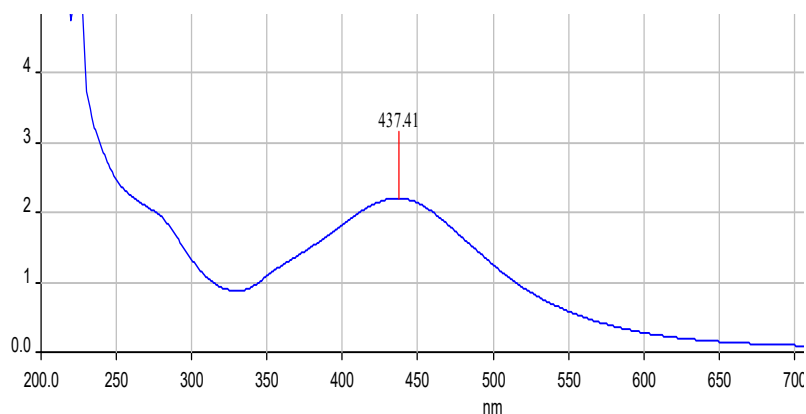


Figure 2

UV–Vis absorption spectra of silver nanoparticles synthesized using *Ficus auriculata* leaf extract.

UV–Vis spectroscopy is one of the most commonly used techniques for structural characterization of silver nanoparticles. The colour change from light to dark brown was observed within 10 minutes of addition. The aqueous extract helped in reduction of silver ions to silver metal and change in coloration indicated the formation of nanoparticles. The electronic transitions involving the Ag^+ ion give rise to absorption bands located between 200 and 230 nm, whereas the electronic transitions of metallic Ag (0) appear in the

250–330 nm spectral range.²⁷ The obtained silver nanoparticles displayed the characteristic surface Plasmon resonance (SPR) band for silver, centered from 425 to 440 nm. Similar type of observations was found during biosynthesis of silver nanoparticles using mangosteen leaf extract in R. Veerasamy et al study.²⁸ There are no peaks found in the region of 335 and 560 nm, indicating the absence of nanoparticle aggregation.²⁹

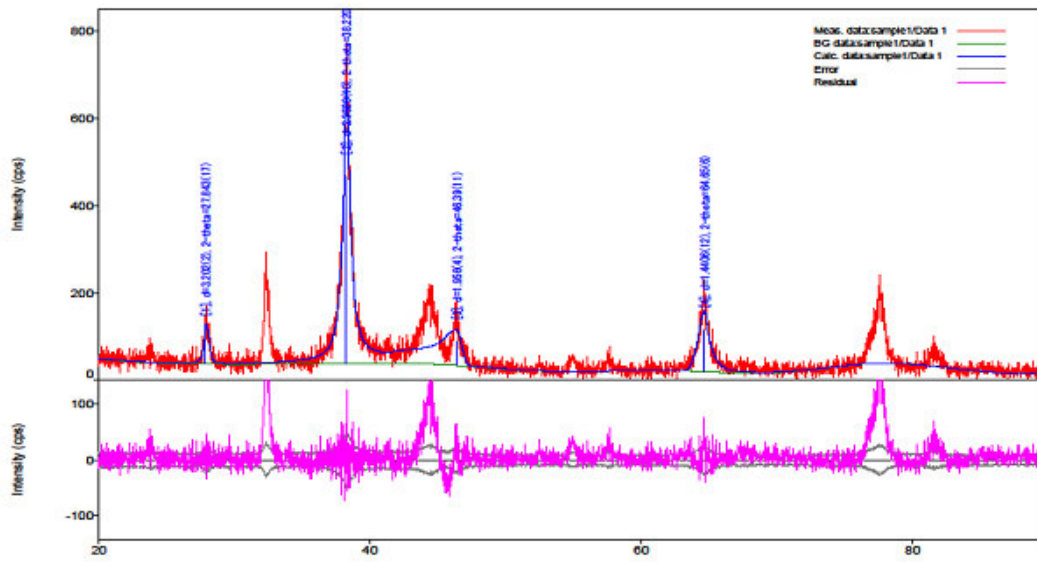


Figure 3
XRD analysis

No.	2-theta	d	Height	FWHM	Int. I	Int. W	Asym. factor	Decay rate factor		Size (ang.)
	(deg)	(ang.)	(cps)	(deg)	(cps deg)	(deg)		eta L/mL	eta H/mH	
1	27.843(17)	3.202(2)	61(14)	0.38(4)	32(3)	0.52(16)	0.3(2)	1.5(2)	0.2(3)	222(25)
2	38.220(17)	2.3529(10)	429(38)	0.55(3)	409(5)	0.95(10)	0.73(12)	1.33(11)	1.07(9)	158(8)
3	46.39(11)	1.956(4)	54(13)	2.2(2)	231(12)	4.3(13)	5.0(19)	1.55(13)	0.0(5)	41(5)
4	64.65(6)	1.4406(12)	95(18)	0.91(8)	116(5)	1.2(3)	1.6(6)	0.01(16)	1.2(3)	108(9)

Table 1
Crystalline size of silver nanoparticles synthesized using *Ficus auriculata*

The XRD patterns of the prepared sample indicate the formation of the silver nanoparticles. Figure 3 clearly shows well defined diffraction peaks at 27.84, 38.220, 46.39 and 64.65 corresponding to the (111), (200) (220) and (311) crystallographic planes of face centered cubic (FCC) silver crystals, respectively (Table 1). The results obtained from the Bark extract of *F. benghalensis* were quite similar to our study.³⁰ The average size of

synthesized nanoparticles can be calculated by using scherrer equation. $D = 0.94 \lambda / \beta \cos \Theta$ Where D = Crystalline size of silver nanoparticles, λ = wave length of x-ray source (1.54 Å), β is full width at half maximum of diffraction peak, k is scherrer constant with a value of 0.9 to 1. Θ is Bragg angle. The average size of silver nanoparticles was found to be 13.2 nm.

SEM Analysis



Figure 4
SEM image of synthesized silver nanoparticles of *Ficus auriculata*

Scanning electron microscope was done using FESEM (Sigma, Carl Zeiss) electron microscope. The analysis was done under the conditions like Extra high voltage, working distance 3.8 mm, high vacuum and room temperature. The observed nanoparticles were mostly

spherical in shape which is in agreement with the findings of silver nanoparticles obtained from leaf extract plant of *Saraca indica* by Perugu et al.³¹ The SEM images showed the presence of both individual and aggregated silver nanoparticles. (Figure 4)

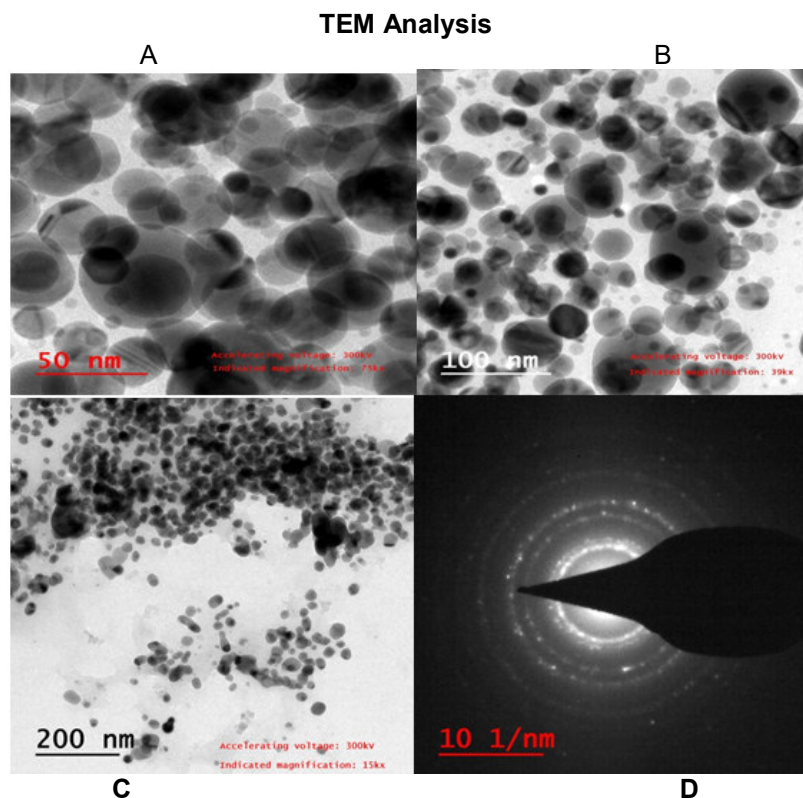


Figure 5

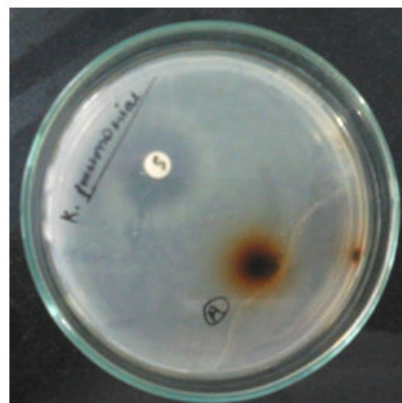
TEM Images of synthesized silver nanoparticles at 50 nm (A), 100 nm (B) , 200 nm (C) and SAED pattern (D)

TEM analysis was done using accelerating voltage of 300 kv .The size and shape of synthesized nanoparticles are clearly observed. It is clear that silver nanoparticles were polydispersed and spherical in shape with average size of 23-67 nm. Sample has two faces in surface morphology and found to be quasi crystal (**Figure 5A-5C**). The selected area electron diffraction pattern (SAED) of the silver nanoparticles is demonstrated in **Figure 5 D**. Just like silver nanoparticle preparation with Gum karaya plant by Venkatesham M et al, the silver nanoparticles in this study are found crystalline,²⁹ as can be observed from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregation. SAED spots

corresponded to the different crystallographic planes of face-centered cubic (fcc) structure of elemental silver.

Antimicrobial Activities

The antimicrobial activity of green synthesized silver nanoparticles was evaluated against both gram positive *B.cereus* and gram negative *K.pneumoniae* bacteria (Figure 6A & 6B). It was observed that green synthesized silver nanoparticles showed clear zone of inhibition (Table-2). Here Streptomycin 25µg/ml was used as Positive Control. We found significant antibacterial activity against both bacteria by synthesized silver nanoparticles.



A (*B. cereus* treated with *Ficus auriculata*) B (*K. pneumoniae* treated with *Ficus auriculata*)

Figure 6
(A&B) Antibacterial studies of synthesized Silver Nanoparticles of *Ficus auriculata*.

S.NO	Organism	Zone of inhibition (mm)	Positive control (Streptomycin-25µg/ml)
1	<i>B. cereus</i>	10.6	22.6
2	<i>K. pneumonia</i>	13.0	18

Table 2
Showing zone of inhibition against bacteria pathogens

CONCLUSION

An ecofriendly and low cost process has been demonstrated in the study for the synthesis of silver nanoparticles by using leaf extract of *Ficus auriculata* plant. Stable silver nanoparticles were synthesized without using any synthetic reagent. The reaction was carried out in environmentally safe solvent water. The synthesized nanoparticles were characterized by different methods such as UV-Vis spectrophotometer, XRD, SEM and TEM. The observations suggest that nanoparticles are crystalline, polydispersed and spherical in shape with 23nm to 67 nm size. Further these green synthesized nanoparticles exhibited

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potential antibacterial activity against *Bacillus cereus* and *Klebsiella pneumonia* bacteria.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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