International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Green Synthesis of Silver Nanoparticles using Indian Tulsi Leaves (Ocimum teniflorum) at Room Temperature & Normal pH and its Study under Different Characterization Tools

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Abstract: ***Silver Nanoparticles synthesis by green route is quite easier using the extract of Indian Tulsi (also known as Holy Basil) leaves, acting as the reducing and stabilizing agent. The process acquired to synthesize nanoparticles (NPs) has been carried over by using very basic and simple laboratorial instruments and techniques. The variety of characterization results are discussed thoroughly. It is found that the synthesized NPs at room temperature and at almost neutral pH value (6.8) are stable to an extent in aqueous form, whereas the synthesized NPs in powdered form show strong stability after several weeks also.***

Keywords: Green Route, Silver Nanoparticles, Holy Basil, Characterization Results, Stability.

1. Introduction

Nano material is an emerging field of material science in today's world. Nano scale can be defined as the size ranging from 1 to 100 nm. Depending upon the dimension in nano scale, the nanoparticles can be divided into four subcategories [1]: 0 D (Spherical small NPs), 1 D (Nanotubes or Nanofibres), 2 D (Nanosheets) and 3 D (Bulk nano structures at macro scale). There are broadly two approaches to synthesize nanoparticles [2]: 1) Top- Down Approach 2) Bottom Up Approach. Top- Down approach is the breaking down of bulk material into nanoscale ranging material. It is a subtractive process. Ball milling, Laser ablation, thermal breakdown are some of its methods [3]. Bottom Up approach is combining of atoms and molecules to form nanoscaled materials. It is a self additive process. Chemical routes, Green synthesis, sol gel method are some of its examples. Among all the methods, Green synthesis is quite appreciable. There are several advantages [4**]** of Green route method, as this method is eco-friendly, having very less bio toxic yielding. This method is considered as one step – one pot process. This method involves the use of plants and living organisms like algae as reducing agent. These natural organisms contain phytochemicals [5] which have been proved as the magnificent precursors to synthesize nanoparticles.

In India, nanoparticles have been being used since time immemorial in the form of Bhashmas and Rashayanas. Our ancient people used to utilize the Bhasmas of Gold, Silver and Copper, as the medicines and supplements. Various compounds in the form of nanoparticles were being used to sharpen the swords or bring unbeaten lustre and brightness on their pots and weapons. The Indians are backgrounded with the Ayurvedic value and using the plants like Holy Basil, Cloves, Cinnamon, Arjuna Bark, etc to synthesize metal nanoparticles renovate our cultural values, in the field of material science.

The synthesis of metal nanoparticles, especially noble metal nanoparticles (Gold, Silver, Platinum, etc) is gaining more importance due to their exotic photoelectric properties[6], higher stability and enhanced permeability and retention effect. In the present study, Tulsi (Holy Basil), a common herb plant found in almost every Indian household has been used as the precursor to synthesize silver nanoparticles. The Tulsi leaves contain necessary phytochemicals like Quercetin **[7**], capable of reducing and capping the metal nanoparticles efficiently.

The synthesized Silver Nps were prima-facie evidenced by the color change of the solution. After 24 hrs, the sample was tested by using UV-Vis spectroscopy in the radiatiton range 300 nm to 800 nm. After 30 days, powdered particles crystallographic size and structure analysis has been done by XRD. To detect the functional groups, responsible for the reduction and capping of silver nanoparticles, FTIR analysis has been done. To check the stability of the nanoparticles in aqueous form, Zeta Potential analysis has been done. The morphology of the powdered sample of NPs have been analysed using FESEM and EDX report has been discussed. The DLS analysis of the aqueous form NPs has been done after 30 days of the sample preparation.

2. Materials and Method

2.1 Materials

1) Silver Nitrate ($AgNO_3$) 99% pure, molecular weight 169.87 gram (Research Lab company Cat.No. 01333) has been bought from local chemical shop.

2) Tulsi also known as Holy Basil (Ocimum teniflorum) leaves have been collected from the university garden premise.
3) Double Distilled water (DDW) has been used as aqueous medium and Ethanol (95% pure) has been used as cleaning and washing agent. Whatman's No. 1 Filter paper (Cytiva company Cat No. 1001-125) has been used for filter purpose.
4) Simple Laboratorial equipments like Hot plate magnetic stirrer, Centrifugal machine (max 15000 rpm), digital pH meter, digital thermometer, digital milligram weighing machine, etc. have been used.
5) All the experiment has been carried out at Room temperature 25° C – 28° C, in dust free and direct sunlight restricted environment.

2.2 Method

All flasks, stirrer, apparatus have been well treated with Ethanol and then DDW.

a) Preparation of Tulsi Extract

20 g of fresh leaves of Holy Basil (Tulsi) is collected and washed twice with DDW. It is smashed with mortar and pestle. The stuff is mixed with 250 ml of DDW and is heated to boil for 10 min. The solution is then left for sedimentation and the liquid part is decanted and filtered through Whatman's No. 1 filter paper. About 100 ml of the Tulsi extract is stored in refrigerator at temp 10° C.

b) Preparation of Silver Nitrate Solution

3 mM concentrated Silver Nitrate solution is prepared by dissolving 500 mg of $AgNO_3$ (Molecular mass – 169.87 gram) in 1000 ml of DDW. The solution is kept at room temperature.

c) Green Synthesis Procedure

250 ml of $AgNO_3$ (aq) is mixed with 25 ml of Tulsi extract (10:1). The solution is again filtered with Whatman's No. 1 filter paper to avoid any bigger particles. Since the concentration is taken higher as compared to the reported literature[8],[9] the reduction occurs very fast. Within half hour the colour change takes place from brown to greenish grey colour as shown in figure 1 & 2. The solution turns into the suspension as the suspended cloudy particles are visible. The pH of the sample measures value 6.8, which is almost neutral. The sample is sent for Uv-Vis and FTIR characterizations and after 24 Hrs, the both analysis is done.

Figure 1: $AgNO_3$ + Tulsi (10:1) at O Hr

Figure 2: $AgNO_3$ + Tulsi (10:1) after 30 min.

The suspension is made to centrifuge at 12,000 rpm for 10 min so that the suspended particles may sediment. Fig. 3 & 4 respectively show the clayey sediment particles which are collected and washed twice with ethanol and made to heat dry at a constant temperature of 50° C, for 15 min. The powdered form is obtained weighing about 50 mg. The powder is preserved in air tight sample tube.

After 30 days of the sample collection, there was the characterization using XRD, FESEM, etc. The powdered sample was in its stable original condition.

Figure 3: AgNPs after centrifugation

Figure 4: AgNPs is washed with Ethanol

3. Results and Discussion

1) *Color Change* – The fast color change from brown to greenish grey is due to the surface Plasmon resonance phenomenon[10], which is an important trait of nanoparticles. The $AgNO_3$ (3mM) and Tulsi extract both have been taken in very high concentration as compared to the reported journals. This may lead to the

fast reduction process even at room temperature and normal pH of 6.8.

2) *Ultraviolet Visible Spectroscopy* – After 24 Hrs of aqueous sample preparation, Uv-Vis analysis is done. The sample in the cuvette taken is too diluted, ranging absorbance value 0 to 1. Figure 5 shows the Uv-Vis Spectra plotted and the peak is obtained at 480 nm, with a good absorbance value of 0.5.

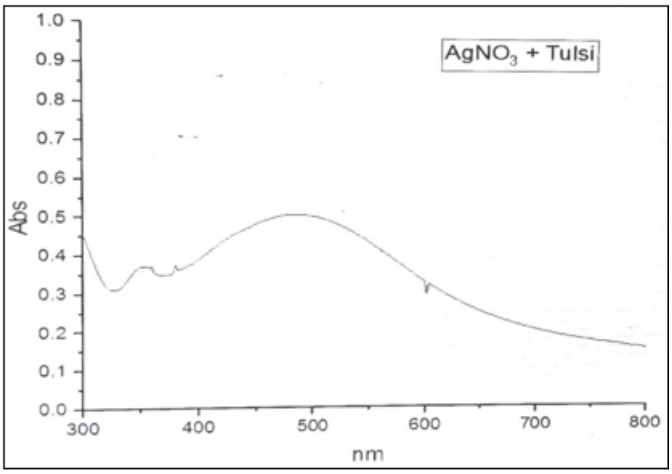


Figure 5: Absorption spectra of synthesized AgNPs

3) *Fourier's Transform Infrared Spectroscopy* – FTIR analysis (Figure 6) of aqueous sample shows that synthesized NPs are enveloped by various phytochemicals having functional groups of alcohols, alkynes, ketones, amides , present in the Tulsi extract. Table 1 presents the details of this analysis. These are responsible for the synthesis and stabilization of the nanoparticles. The result has been matched from the standard data reference.[**11]**

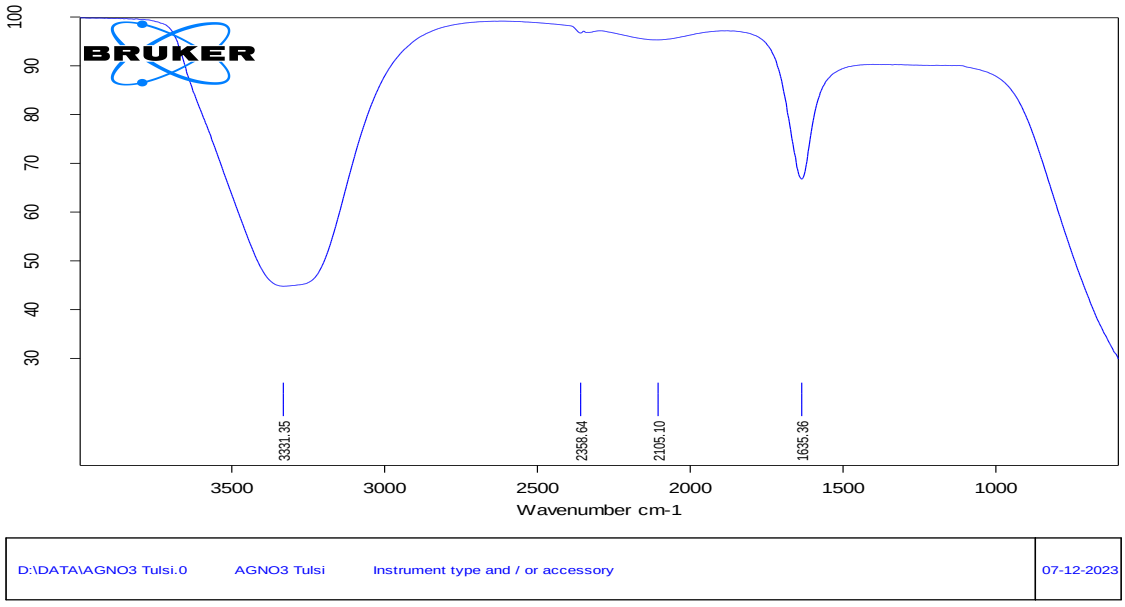


Figure 6: FTIR analysis of $AgNO_3$ + Tulsi (10:1) aq.

Table 1: Details of Functional Groups at different peaks

Peak Wavenumber (cm^{-1})	Absorption %	Intensity of Peak	Shape of the peak	Functional group *From Standard Reference*
3331.35 cm^{-1}	60 %	Strong	Broad	(Hydroxy group) Normal Polymeric OH stretch
2358.64 cm^{-1}	5 %	Weak	Sharp	C=O (Carbonyl group)[#]
2105.10 cm^{-1}	8 %	Weak	Broad	$C \equiv C$ Terminal Alkyne
1635.36 cm^{-1}	30 %	Medium	Sharp	$CONH_2$ Amide

4) *X-Ray Diffraction Analysis* - XRD analysis of the powdered sample is done after 30 days of the sample preparation. The powdered sample shows modest crystallite size indicated by the large number of diffraction peaks. Figure 7 shows the peaks at 38.20º, 44.36º, 64.56º, 77.50º which are linked with the diffraction lattice planes of (111), (200), (220) and (311) respectively, which is in the good agreement with the previous reports [12]. Silver XRD pattern was found

to match with the standard face centred cube (fcc) crystal lattice XRD card (JCPDS file No. 04-0783).

In addition to these four peaks of FCC silver particles, there are some additional peaks (28.04º, 32.36º, 46.31º, 55º, 57.6º) observed [13] which may be due to the unreduced $AgNO_3$. Another report [14] suggests that these peaks may arise due to the presence of some other inorganic compounds in very small quantity.

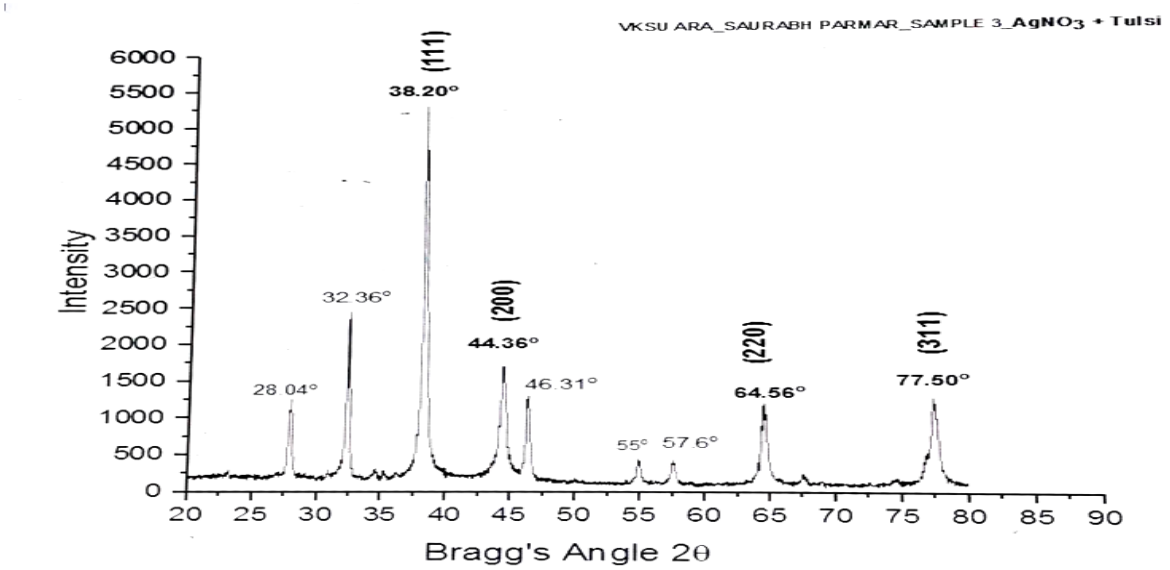


Figure 7: XRD diffraction Pattern of Powder AgNPs

Crystal size has been calculated with Debye Scherer equation [15] by using the Table 2 data. The average crystal size is about 16 nm.

Table 2: Crystal diameter is calculated by using the following XRD mathematical data

Peak Angle 2Θ (°)	Intensity	Θ (°)	Cos Θ	FWHM(°)	FWHM (rad) β	Crystal Diameter (nm)
38.20 °	5295	19.10°	0.94494	0.41	0.0071	20.12 nm
44.36°	1696	22.18°	0.92601	0.64	0.0111	13.13 nm
64.56°	1200	32.28°	0.84544	0.51	0.0088	18.14 nm
77.50°	1275	38.75°	0.77988	0.68	0.0118	14.66 nm

5) *Field Emission Scanning Electron Microscope (FESEM) and Energy dispersive X-ray spectroscopy (EDX) Analysis* – The scanning has been done at 4 level magnifications i.e. 10 KX, 25 KX, 50 KX and 100 KX, after 3 months. The fig. 8 to 11 shows the morphology of the synthesized NPs at different magnifications.

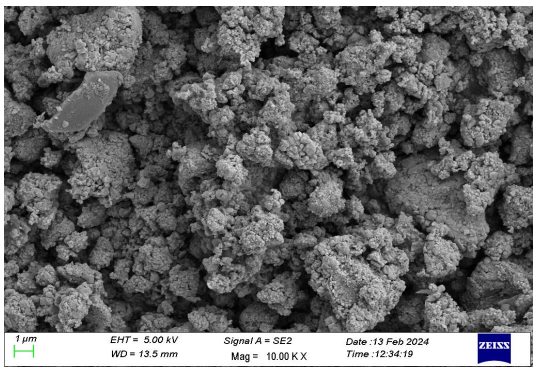


Figure 8: FESEM at 10 KX magnification

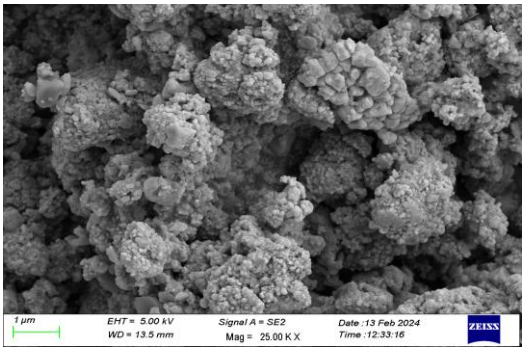


Figure 9: FESEM at 25 KX magnification

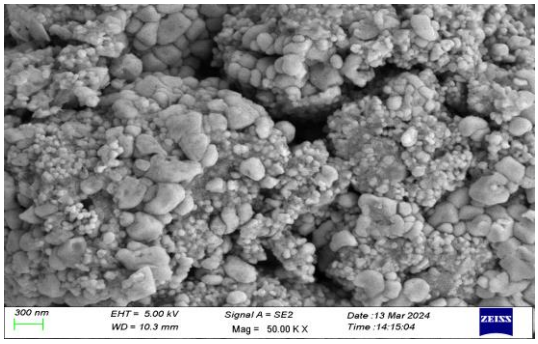


Figure 10: FESEM at 50 KX magnification

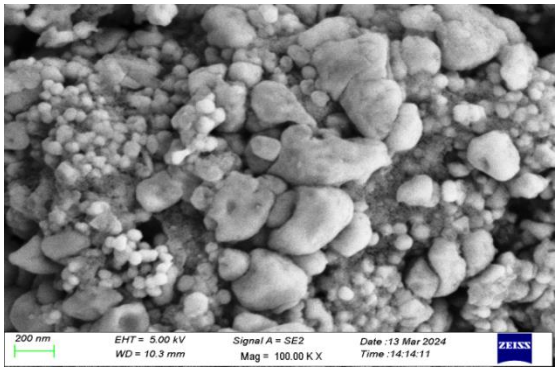


Figure 11: FESEM at 100 KX magnification

.EDX report (Figure 12) presents the elemental analysis of the sample. Here it suggests that the sample has Ag (Silver) element in abundance (74.21 %).

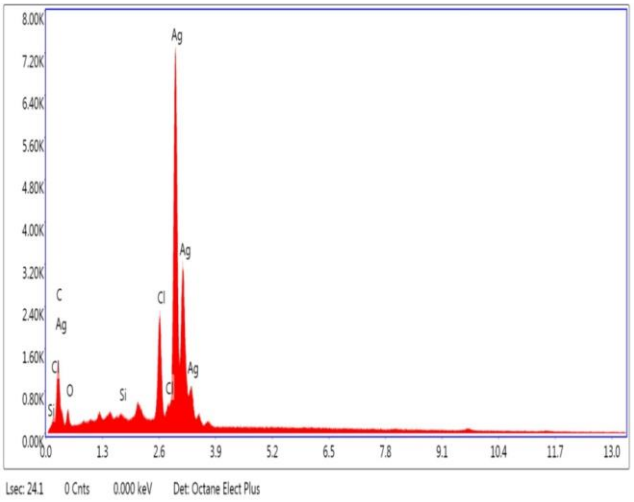


Figure 12: EDX analysis of the Powder AgNPs

6) *Zeta Potential Analysis:* The analysis has been done after 30 days of the sample preparation. The aqueous sample even after many days comparatively shows a good stability having negative zeta potential -18.08 mV (Figure 13). The reported journals suggest that it is an incipient coagulation [16] (initial phase of agglomeration). However, it may be due to the particles density greater than dispersant, and in that case it sediments showing coagulation.

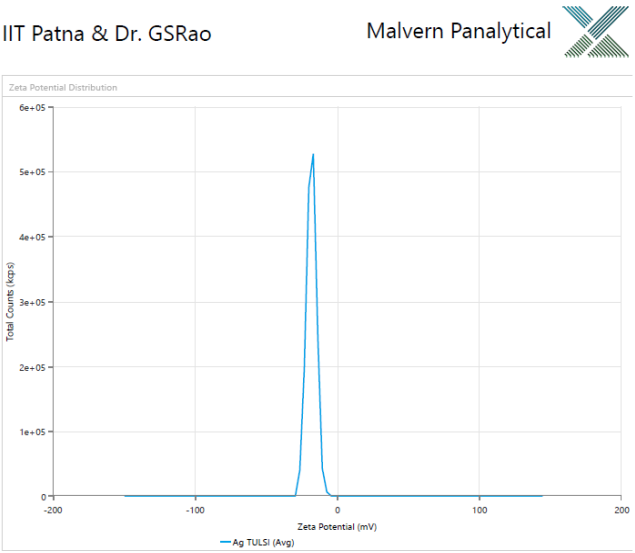
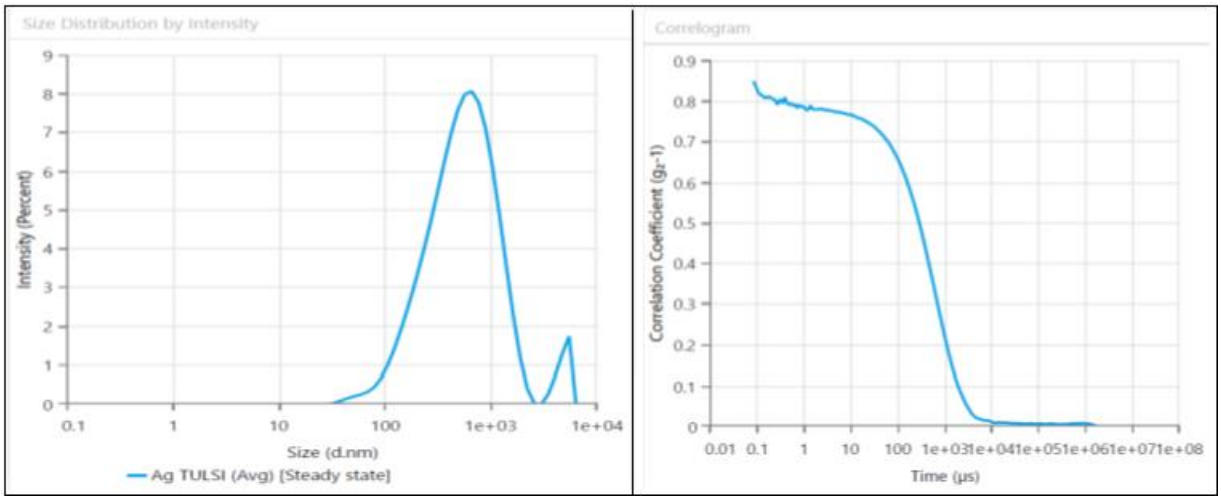


Figure 13: Zeta Potential Analysis

7) *Dynamic Light Scattering Analysis (DLS)*– DLS of the aqueous sample has also been done after several days. It is an important tool to estimate the particle size in aqueous form. The particle size (Figure 14) in the aqueous state is nearly 434 nm, which is clearly evidencing that the aggregation of nanoparticles exists in the suspension, after several days. Thus, the measured value of the particle size is bigger, as the aggregation is treated as the single particle. In the correlogram (Figure 15), the y-intercept at nearly 0.9 shows a very strong positive correlation [17]. The slope of the gradient is large indicating that there is moderate polydispersity [18]. The polydispersity index 0.44 in Table 3 is marking this fact.

Table 3: DLS Particle Size Analysis Report Table

Sample Name:	Ag TULSI (Avg)		
Project Name:	SAURABH PARMAR		
Date and Time:	06 February 2024 16:01:30		
Type:	Size	**Result Source:**	Average
Cell Name:	DTS0012	**Temperature (°C):**	25.01
Material Name:	Default Sample1	**Dispersant Name:**	Water
Material RI:	1.33	**Dispersant RI:**	1.33
Material Absorption:	0.01	**Dispersant Viscosity (cP):**	0.887
		Dispersant Dielectric Constant:	78.5



Statistics Table

Name	Mean
Z-Average (nm)	434.5
Polydispersity Index (PI)	0.4485
Intercept	0.878
Fit Error	0.003272
In Range (%)	93.98

Figure 14: DLS Particle Size Analysis and **Figure 15:** DLS Correlogram

4. Conclusion

Green synthesis of silver nanoparticles using Holy Basil leaves (Ocimum teniflorum) is fast and simple process, even at room temp and normal pH. The process is environment friendly and requires less sophisticated equipments. The variety of characterization tools analysis evidence that the particles in powdered form as well as in aqueous solution are quite stable even after several weeks. The powdered NPs show a robust stability in comparison to the aqueous one. These stable Silver NPs have diverse applications. These can be used as antibacterial medicines and antimicrobial water purifiers due to their excellent properties of penetrating the cell wall and attacking the ribosomes [19]. The plasmonic properties of AgNPs can be used in making bio-sensing & imaging materials [20]. The AgNPs can effectively show cyto-toxic effect, thus these green synthesized AgNPs may be explored to be used in drug preparation for cancer disease.

Acknowledgement
The author is obliged to Dr. Anil Kr. Singh (Professor, Deptt of Physics, VKSU Ara) as a mentor and the whole PG department of Physics, VKSU Ara as a collaborative institution, where the experiments have been carried out. In addition, the author would like to thank SAIF

Centre and ME department of IIT Patna (Bihta Campus, Bihar) where the characterizations have been done.

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Author Profile

Saurabh Parmar completed his Graduation from Maharaja College Ara Bihar India and received the M.Sc. degree in Physics from P.G. Deptt. Of Physics, Veer Kunwar Singh University Ara Bihar India. As a young research scholar, he has been pursuing his Ph.D. research study under the supervision of Dr. Anil Kr. Singh (Professor, PG Deptt of Physics, Veer Kunwar Singh University) since year 2021. At present (since Feb 2024), Saurabh Parmar is also serving as the PGT Physics Govt. Teacher under the Education Department, State Govt. of Bihar, India. He is attributed to the blessings of his eternal Grandfather- "Shyam Ji Singh".