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# Characterization of Green Synthesized Silver Nanoparticles Using *Azadirachta Indica* (Neem) Leaf Extract

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## ABSTRACT

*There is a continuously growing demand for metal nanoparticles due to their wide spread applications in medical health care sector. Eco-friendly synthesis of these nanoparticles on the contrary has also become a major constrain for nanotechnology nowadays against the health hazardous chemical technology popularly used for the purpose. In the present paper, one such eco-friendly but economical way developed in the synthesis of silver nanoparticles by using extract of Azadirachta Indica leaf; commonly known as 'Neem' belongs to the family Meliaceae, is described. Efficacy of this green element as a reducing and capping agent in the synthesis of silver nanoparticles (AgNPs) from 0.1 gm. silver nitrate ($AgNO_3$) has been investigated. The resulting AgNPs was characterized using UV–Vis, DLS, FTIR, and EDS. Their antibacterial activity important for health care sector was confirmed using Agar well diffusion test against E.Coli and S.Aureus.*

*Silver nanoparticles synthesized within 24 hours of incubation period have shown an absorption peak at around 400 nm in the UV-visible spectrum. Increase antimicrobial properties were also reported for this newly synthesized colloidal. This innovative green AgNPs synthesis route is thus found equivalent to popular chemical route in all positive aspects, like speed, simplicity and effectiveness but remain aloof from health hazardous outcomes and economical.*

***Keywords-Agar well diffusion, Azadirachta Indica, biosynthesis, Neem methanol extract and Silver colloidal solution***

## I. INTRODUCTION

Nanotechnology is recognized as an emerging empowering technology for the 21$^{st}$ century, in addition to the already established areas of information technology and biotechnology. This is because of the scientific convergence of physics, chemistry, biology materials and engineering at nanoscale, and of the importance of the control of matter at nanoscale on almost all technologies. Nanoparticle manufacturing is an essential component of nanotechnology because specific properties can be realized either at the nanoparticle, nanocrystals or nano layer level, and assembling of pioneer particles and related structures is the most generic route to generate nanostructured materials [1].

The most prevalently used nanoparticle can be prepared either by nanoparticle synthesis or by processing nanomaterial into nanostructured particles. Synthesis of nanoparticle can be carried out by either of chemical,

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physical and biological methods [2]. Chemical method is advantageous amongst all due to its shortest period of time used in synthesis. Conflicting this, chemical reagent used are expensive and highly toxic on the account of by-products generation which are not eco-friendly. Physical methods on the contrary are not that much harmful to the environment. Nevertheless, they demand high amount of energy, occupy large space, expensive and time consuming. In comparison biological method does not generate any toxic by-products thereby environment friendly. It also follows shorter production route, having greater product durability and availed from natural resources exist in abundant varieties and quantities. Thus on an aggregate the system leads to a low cost, eco-friendly as well as reliable technique [3]. Over the past several years, plants, algae, fungi, bacteria, yeast and viruses have been used for production of inexpensive, energy-efficient and eco-friendly metallic nanoparticles [4].

The major advantage of nanoparticle synthesis using plant extracts is that they are easily available, safe, and nontoxic in most cases. The added advantage of this resource in Nano synthesis is the ready availability of natural capping agents from the plants itself. They have a broad variety of metabolites that can aid in the reduction of ions, and thereby found more quickly than microbes in the synthesis and capping agent [4].

## II. MATERIALS

**Chemicals:**

- Silver nitrate and methanol
- *Azadirachta Indica* (Neem) leaves.
- Distilled water
- Cultures Dehydrated Luria broth and Nutrient agar media

## III. METHODS

The research has involved preparation of Neem Methanol Extract (NME), AgNPs Colloidal and evaluation of AgNPs characteristics

Preparation of Neem Methanol Extract (NME) and Silver Nitrate solution (SS)

Indian medicinal plant Neem was selected from garden, on the basis of cost effectiveness, ease of availability and medicinal property. Neem powder is prepared as shown in fig 1. 25 gram Neem powder was added in 250 ml of Methanol and given constant stirring at 250 rpm with magnetic stirrer at room temperature for 30 min. Finally, the solution is filter twice by whatman filter paper. The extract with 10 percent concentration so obtained have typical dark greenish tint. In a thoroughly cleaned vessel, 0.1 gram silver nitrate salt was taken and dispersed in 100 ml DW. This has produced 0.1% percent concentration of Silver nitrate solution.

| | |
|---|---|
| I. Fresh and Healthy Leaves Plucked from the tree and washed well with distilled water |  |

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| | |
|---|---|
| II. Neem leaves are oven dried |  |
| III. Dry Neem leaves are grinded well into Mixer |  |
| IV. Fine Neem powder obtained on sieving. |  |

**Fig 1: Neem powder extraction process**

### 3.1.Synthesis of Silver nanoparticles

Series of pilot trials were conducted and broadly can be bifurcated into two sets. In first set quantity of Neem Methanol Extract (NME) was varied by keeping silver nitrate salt quantity constant to get its optima. Whereas in the second set quantity of silver nitrate salt was varied by keeping optimum add on of Neem Methanol extract defined in first set The best colloidal quality was decided on the basis of same criterion, which encompassed presence of silver particles in nano form along with their uniform distribution. Neem Methanol Extract (NME) of 10 percent concentration was diluted by the addition of 50 ml DW before the addition of Silver Nitrate (SS) solution in the mixture to condense reduction potential of NME. Addition of distilled water for dilution purpose was well defined on the basis of series of empirical trials conducted. This was determined on the basis of expert's visual analysis and UV-Vis.spectroscopy.

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**Table 1: Experimental set up – I**

| Set no | sample | Neem Methanol Extract (ml) |
|---|---|---|
| A | S-0.3 | 5 |
| B | S-1.4 | 25 |
| | S-1.3 | 20 |
| | S-1.2 | 15 |
| | S-1.1 | 10 |
| | S-1.0 | ***5*** |
| C | S-1.0 | 5 |
| | S-2.1 | 4 |
| | S-1.5 | 3 |
| | S-2.0 | 2 |
| | S-1.6 | ***1*** |
| D | S-1.6 | 1 |
| | S-1.7 | 0.75 |
| | S-1.8 | 0.5 |
| | S-1.9 | ***0.25*** |

**Table 2: Experimental set up-II**

| Sample | $AgNO_3$ Salt solution (ml) |
|---|---|
| H-0.1 | 1 |
| H-0.2 | 2.5 |
| H-0.3 | 5 |
| H-0.4 | 7.5 |
| H-0.5 | 10 |
| H-0.6 | 12.5 |
| H-0.7 | 15 |
| H-0.8 | 17.5 |
| H-0.9 | 20 |
| H-1.0 | 22.5 |
| H-1.1 | 25 |
| H-1.2 | 27.5 |
| H-1.3 | 30 |
| H-1.4 | 32.5 |
| H-1.5 | 35 |
| H-1.6 | 37.5 |
| H-1.7 | 40 |
| H-1.8 | 42.5 |
| H-1.9 | 45 |
| H-2.0 | 47.5 |
| H-2.1 | 50 |

### 3.2.Characterization of silver nanoparticles

Shimadzu UV-Vis Spectrophotometer (UV-2450) is used for the UV-Vis spectral analysis to reveal presence and concentration of nanoparticle. To determine the average size of synthesized silver nanoparticles Dynamic light scattering (Brookhaven 90Plus particle size) was used. Shimadzu FTIR spectrometer (FTIR – 8400 S) was used to record the FTIR spectra for chemical composition of colloidal solution. The elemental analysis of AgNPs colloidal solution was performed in this test using the same Scanning Electron Microscope (SEM) (Model JSM-5610 LV, Version 1.0, Jeol Japan). The antibacterial activity was checked against both Gram positive bacteria and Gram negative bacteria according to Test Methods as per AATCC - 25922 for E.Coli and AATCC- 29213 for S.Aureus (Agar well diffusion method). Grading the performance of optimum AgNPs

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colloidal solution for antibacterial activities in comparison with commercially successful Ciprofloxacin chemical.

## IV. RESULTS AND DISCUSSION

### 4.1.Confirmation of AgNPs Formation

The colour of colloidal solution was changed from green to transparent brownish shade on the successful formation of AgNPs as shown in figure 2; on the reduction of macro size silver metal particle size to nano form. Formation of AgNPs in the colloidal solution was instantly realized by this visual colour change test. Such subjective confirmation for synthesized silver nanoparticles was objectively confirmed later on by UV–Visible Spectroscopy. This technique takes absorbance values of silver nanoparticle solution into account. According to Mathur [5] presence of silver nano particles in the colloidal is indicated in UV–Visible Spectroscopy by the maximum absorbance value (λ max) wavelength in the visible range of 400–500 nm.

Samples S 1.5, S 1.6, S 1.7, S 1.8, S 1.9, S 2.0 have shown the peak value well within the range but samples S 1.4, S 1.3, S 1.2, S 1.1 and S 1.0 have resided beyond preferable SPR band. It can be appreciated that wavelength for all the samples has fallen well within the band range of 400-500 nm irrespective of $AgNO_3$ (SS) concentration variant. Shallow peak made up of zigzag trend lines was observed in SPR band while working with too high concentration of AgNO3 (SS) for samples H 0.9. The zigzag lines are showing instability of sliver nanoparticle size.Since at high concentration, instable as well as too high number AgNPs has resulted in agglomeration and thereby formation of oversized silver nanoparticle. This gist was realized even during visual test, where initially brownish solutions were turned down to black with increase in time dwell [fig 3and fig 4]. This colour change of colloidal solutions has substantiatedaggregation of initially formed AgNPs in high concentration to undesirable oversized particle.

However, going in agreement to visual judgement amongst all, sample H-0.7 and H-0.9 have executed highest and smooth peak; indicating not only formation of AgNPs but their long term stability.

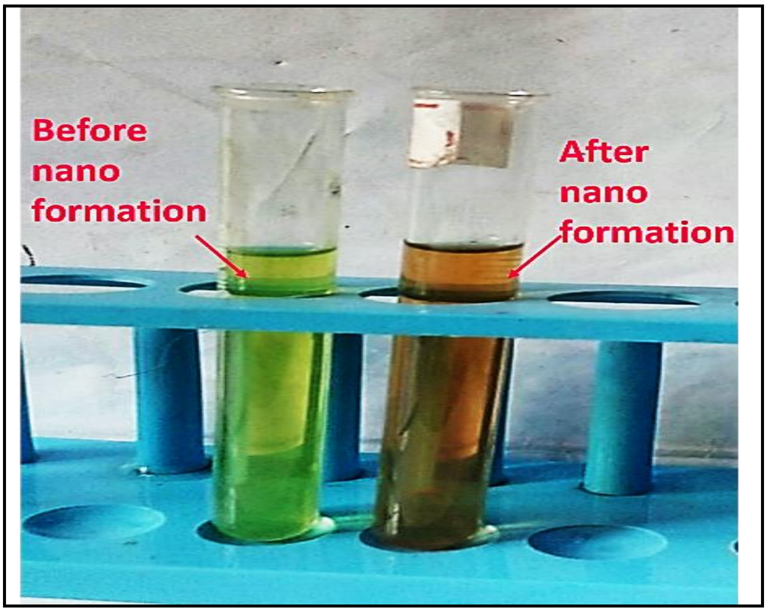


**Fig 2: Color testing for AgNPs colloidal solution**

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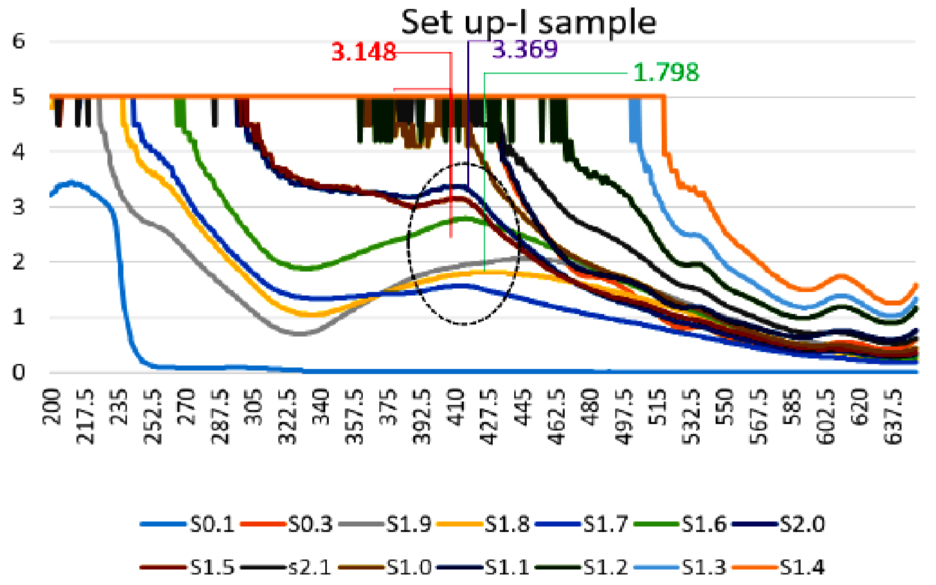


**Fig 3: UV-Visual spectra for setup-I samples**

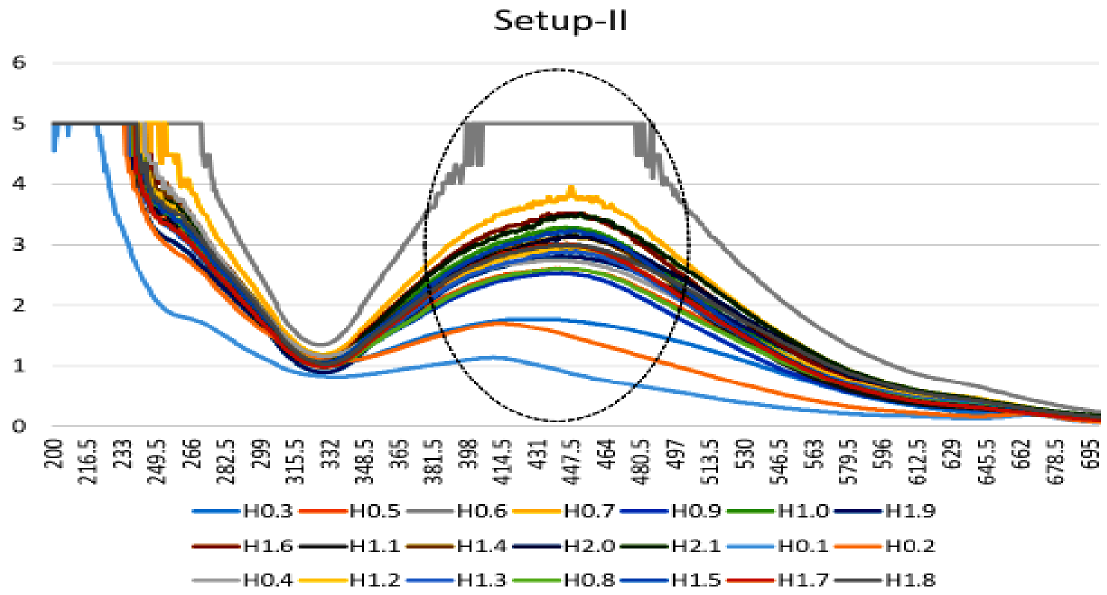


**Fig 4: UV-Vis spectra. Of setup-II**

### 4.2. Antibacterial Qualitative Assessment of AgNPs colloidal solution

Mean width of Zone of Inhibition (ZOI) was measured during Antibacterial qualitative evaluation after 24 hours contact with bacteria for the samples under study and given graphically [figure 5]. Average width Zone of Inhibition (ZOI) represents clear zone without bacterial growth. Tested colloidal sample's ZOI were then compared to sort out one with optimal antibacterial quality.

Hany Yehia [6]has found high activity of NME based colloidal sample against S.Aureus and no effect against E.Coli. But going against that pure NME based colloidal sample in the present study was failed in antibacterial qualitative assessment and executed zero value for mean zone of inhibition for both E.Coli and S.Aureus [figure 6]. This can be due to low quantity about 0.1 ml of 10 percent concentration NME was used in synthesis.

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Silver is a well-established antibacterial agent [7], so good degree of antibacterial activity is likely for only $AgNO_3$ (SS) based solution [sample SS]. However, its antibacterial activities were rated lower than AgNPs colloidal samples. This has mainly attributed to the higher specific surface offered by nano particles in comparison with macro form of the same metal which has played a major role in gearing up antibacterial activity. Samples H-0.7 and H -0.9 were shortlisted during visual test for better AgNPs formation, have also shown optimum zone of inhibition for both E.Coli and S.Aureus bacteria [figure 5]. Hence comparatively low quantum of AgNO3 was used in the synthesis of sample H-0.7, although belonging to equal grade to H -0.9 in terms of performance, it was short listed as an optimum colloidal sample.

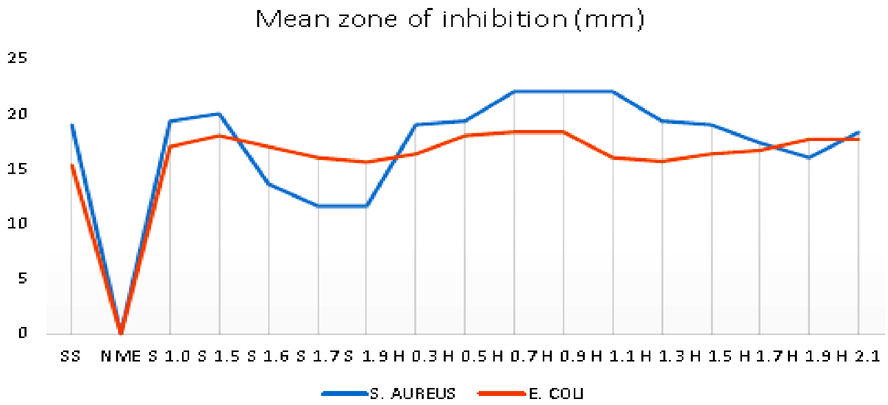


**Fig 5: Antibacterial result of AgNPs, NME and $AgNO_3$ (SS) solution**

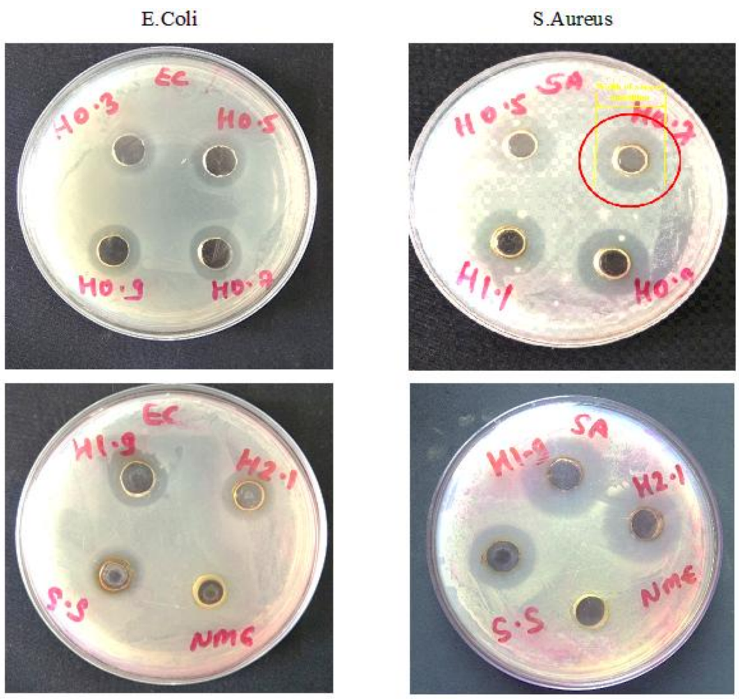


**Fig 6: Antibacterial activity of AgNPs colloidal solution, $AgNO_3$ (SS), NME**

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### 4.3. Analysis of AgNPs size using DLS

Dynamic light scattering (DLS)has measured the mean effective diameter and most of synthesized AgNPs were in the range of 100 nm.The PDI is an indication of variance in the sample, a low PDI usually less than 0.2 indicates that the sample is monodispersed. Monodisperse means as particle contain of uniform size [8].

Optimum defined colloidal sample H-0.7, was verified for nano agglomeration by this test [figure 7]. It can be observed that sample H 0.7 possesses silver nanoparticles monodispersed mixtures in the range 97.8 to 100.7nm. The average size of this synthesized silver nanoparticles using Neem leaves is around 98.8 nm, which is well within the predefined nano particle size. The average poly diversity Index (PDI) is 0.187 with standard error of 0.9, graded as"uniform" for the size of AgNPs [9].

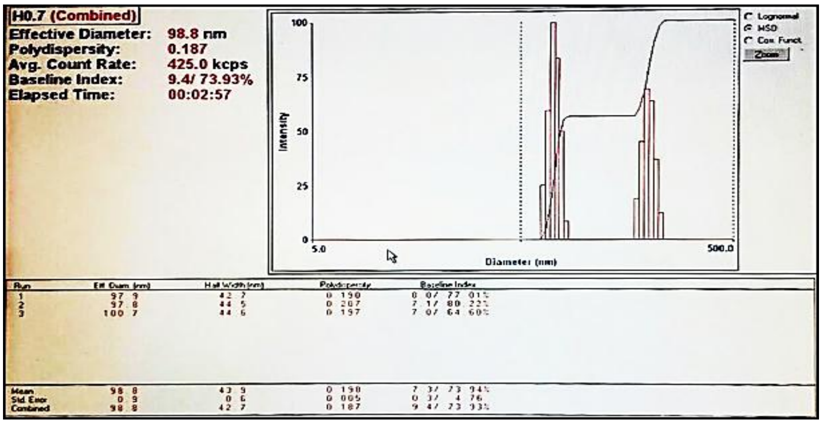


**Fig 7: DLS Histograms of AgNPs sample H 0.7**

### 4.4. Analysis of chemical composition using FTIR

FTIR spectra reveals the composition of solid, liquid and gases. Infrared spectroscopy is an important technique in organic chemistry to identify the presence of certain functional groups in a compound or a molecule of any product. To know the individual chemical molecule or compound (structures) produces different spectral fingerprints. This instrument generates the spectra with patterns that provides the structural insight, to know the functional group present in the molecule or a compound. The spectra pattern obtained for AgNPs sample H 0.7 through FTIR analysis has been shown in Fig 8. It can be seen from figure the range of first broad spectra lies between 3456.55 cm-1 to 3383.26 cm-1. This peak is attributed to the O–H stretching modes of vibration in hydroxyl functional group in alcohols and N–H stretching vibrations in amides and amines. Thus first peak formation is due to the N-H stretching vibration of group NH2 and OH, represents overlapping of the stretching vibration caused on the account of Distilled water and *Azadirachta* Indica leaf extract molecules in the present study [10].

The second peak have value ranging from 2349.38 cm-1 to 2308.87 cm-1, attributed to Si-H stretching of Silane and P-H stretching of Phosphine. So the second peak identified Phosphine, the typical stabilizer and mainly prevents the agglomeration for nanoparticle as per finding of Pastoriza [11] whereas Silane is a coupling agent as per Matthias Epple[12]. The third peak ranges from 1653.05 cm-1 to 1633.76 cm-1 and attributed to amide C=O stretching and alkene C=C stretching. Fourth peak has shown a value of 1384.94 cm-1 corresponds to Alkyl halide, whereas fifth peak has shown a value of 675.11 cm-1 corresponds to Alkene and Alkyl halide for =C-H bending and C-Cl stretch.

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All the three observed peaks have thus endorsed in excess presence of flavonoids and terpenoids in the Neem plants extract [13]. Flavanones and terpenoids are the constituents present in the Neem leaf broth which are responsible for stabilizing the formation of nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids [14].

It is confirmed from the FTIR analysis that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal, indicating that the proteins could possibly from the metal nanoparticles for capping of silver nanoparticles and to prevent agglomeration so as to stabilize the medium. This advocates dual functioning of the biological molecules, viz; formation and stabilization of silver nanoparticles in the aqueous medium.

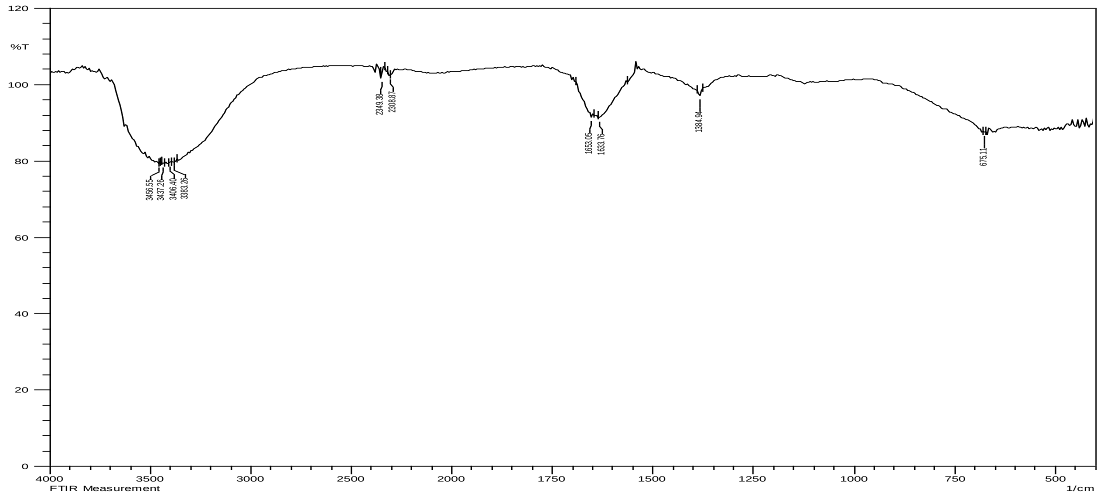


**Fig 8: FTIR spectrum of AgNPs Sample H 0.7**

**4.5. Elemental analysis of Colloidal solution**

EDS test result of sample H-0.7 is shown in Figure 9. It shows the energy dispersive spectrum of the synthesized nanoparticles andsupports the presence of silver as the ingredient element. Metallic silver nanoparticles generally show a typically strong signal peak at 3 keV, due to surface Plasmon resonance[15].Going in line with this EDX spectra of the synthesized silver nanoparticle from Neem leaf extract has confirmed the presence of elemental silver in the sample by executing peaks around 3.0 keV correspond to the binding energies of AgL. Quantitative analysis has proved 6.56% silver contents in the examined samples (the Mass% of silver in the sample is 6.56 and Atom% is 1.32). The presence of elements such as Ag, O, Na, K, Cl, Ca, Si and Mg are clearly visible in figure.

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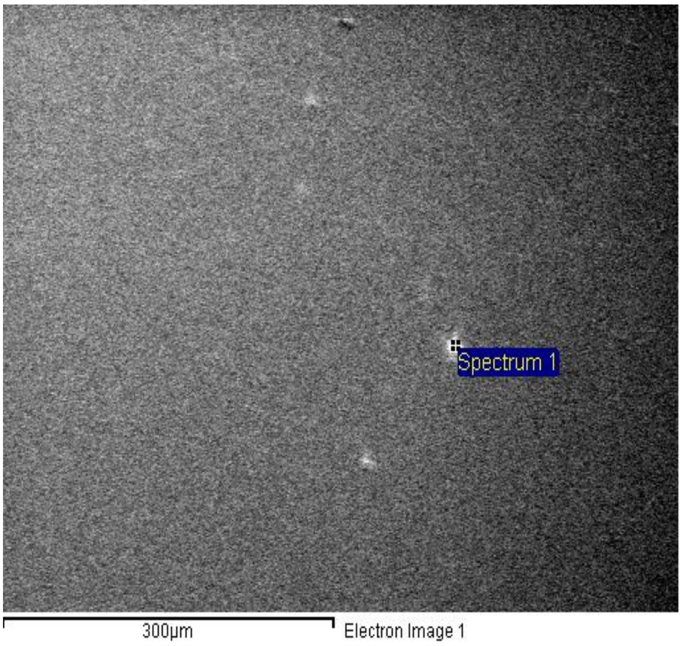
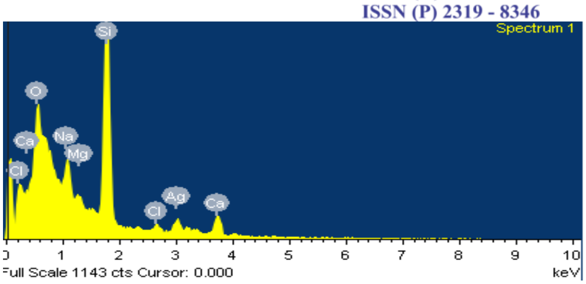



Electron Image




Elemental Graph

| Element | Weight% | Atomic% |
|---|---|---|
| O K | 43.79 | 59.64 |
| Na K | 8.92 | 8.45 |
| Mg K | 2.45 | 2.20 |
| Si K | 32.13 | 24.93 |
| Cl K | 1.47 | 0.90 |
| Ca K | 4.69 | 2.55 |
| Ag L | 6.56 | 1.32 |
| Totals | 100 | |

**Fig 9: EDS result of colloidal solution H 0.7**

**1.1**Comparative Performance Evaluation of AgNPs with Commercial Antibiotic for Antibacterial Activity

Ciprofloxacin is the well-established antibiotic pharmaceutical chemical. An experiment was thereby extended to evaluate performance of the innovative green synthesized antibiotic colloidal solution antibacterial effect against the commercially successful antibiotic results by using Agar well diffusion method.

Test results were recorded with high resolution camera for both sample H-0.7 and different concentration of ciprofloxacin to obtain their respective ZOI values.These values were than evaluated to obtain MIC (minimum inhibitory concentration) of AgNPs colloidal solution with respect to ciprofloxacin performance.Here MIC means minimum concentration of the solution that regularly inhibits the growth of a bacterium in vitro-abbreviation [16].

It was observed that antibacterial activities of 0.1µgm/ml concentration Ciprofloxacin solution against S. Aureus & E. coli bacteria respectively is almost similar to those observed for sample H-0.7 [figure ]. Thus MIC for selected H-0.7 AgNPs colloidal solution is 0.1µgm/ml Ciprofloxacin solution. Accordingly different grading can be worked out for AgNPs solution used in different sectors of the health care articles.

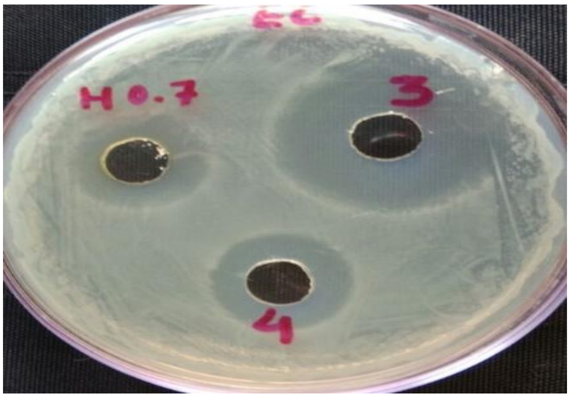



**Fig 10: Antibacterial activity of antibiotic and silver colloidal solution**

## V. CONCLUSION

Silver nanoparticles (AgNPs) were successfully obtained from bio reduction of silver nitrate solutions using Neemleaf extracts. In summary, visual observations, UV–Vis, DLS, FTIR and EDS techniques confirmed the formationof silver nanoparticles by Neem leaf extracts. This work indicates that Neem leaf extract had a good valuablepotential in the future for production of silver nanoparticles. Hence, due to their benign. The synthesised silver nanoparticles showed efficient antimicrobial activities against both E. coli and S. Aureusand stable nature theseSilver nanoparticles (AgNPs) may be well utilized in industrial and remedial purposes Benefits of using plant extract for synthesis is that it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer productThis eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for synthesis of silver nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opto-electronics and medical devices in near future.

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