# "Synergistic Antibacterial Effects of Silver Nanoparticle formed by Cyamopsis tetragonoloba seed extract with Combinations of Antibiotics"

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**Abstract -** By combining silver nanoparticles with the antibiotic streptomycin and Gentamycin, which target Escherichia coli, the antibacterial qualities of Cyamopsis tetragonoloba seed extract are improved. The use of nanoparticles as antimicrobial agents is a promising way to address the problem of multidrug-resistant (MDR) bacteria; this strategy is preferred because microbes are less likely to develop resistance to nanoparticles and because the particles are naturally effective against bacterial cells. Cyamopsis tetragonoloba seed extract and silver nanoparticles were shown to have a minimum inhibitory concentration (MIC) of 15 mm,12 mm and 10 mm at different concentration against the bacterial strain. When silver nanoparticles were added to each studied antibiotic, their effectiveness was noticeably increased compared to when they were administered alone 36 mm and 22 mm respectively. Silver nanoparticles and their combinations' characterisation with Zeta Potential, Transmission Electron Microscopy (TEM), UV-visible spectroscopy, and Fourier Transform Infrared (FTIR) spectroscopy were used to characterise silver nanoparticles and their combinations. The results show that solutions with larger silver nanoparticle concentrations have better antibacterial efficacy. Furthermore, when combined with the antibiotic streptomycin and gentamycin, AgNP therapy showed synergistic benefits.

**Keywords:** Cyamopsis tetragonoloba, Fourier Transform Infrared, synergistic, Characterisation

## I. INTRODUCTION

New kinds of nanoparticles are being developed as a result of the fascinating scientific topic of nanotechnology, which is also producing novel applications in biotechnology and nanomedicine. Physical, biological, and chemical approaches can all be used to create nanoparticles, however the latter two are frequently shunned because to their high expenses, poor yields, and usage of hazardous reducing agents. Plants and microbes are used in biological synthesis techniques to create nanoparticles (Ping Li et al., 2005). However, because of the release of toxic chemicals, using microbes to synthesise nanoparticles is frequently discouraged (Danielle McShan et al. 2015).

On the other hand, plant-based synthesis has attracted attention because to its low cost, easy accessibility, ease of management, environmental friendliness, therapeutic qualities, and lack of need for a sterile environment (Dua B and Jiang H. V 2007). Many bacteria are now able to live and proliferate even when exposed to standard antibiotics because they have evolved resistance to these drugs. Antibiotic resistance problems are also associated with biofilm development. The biofilm matrix, a three-dimensional, gel-like, highly hydrated, and locally charged environment, is frequently how bacteria stick to surfaces (Zyang et.al.,2015).

Infections may arise as a result of these bacteria's attachment to different organs. Bacterial antibiotic resistance is a growing worldwide health concern that is acknowledged as a medical issue that increases rates of morbidity and death, leading to longer hospital stays, higher expenses, and worse outcomes (Montserrat Lopez-Carrizales et al., 2018). According to Guoqing Wan et al. (2016), the rate at which bacteria are developing resistance to current antibiotics is actually faster than the rate at which new antimicrobial molecules are being developed. Unfortunately, it is still quite difficult to find novel bacterial targets for the development of safe and efficient classes of antimicrobial medicines (Habash MB et.al., 2014).

Although the combination of silver nanoparticles (AgNPs) with antibiotics has shown improved efficiency against some bacteria, its impact on multidrug-resistant E. coli remains unclear (In-sok Hwang et al., 2016). AgNPs and streptomycin antibiotics, which were chosen for their different chemical structures and unique antibiotic modes of action, are evaluated and compared for their antibacterial efficacy in this study. various interactions between these antimicrobial compounds and AgNPs might provide various synergistic effects (Guoqing Wan,et. al., 2016).

## II. MATERIALS AND METHODS

**1. Preparation of sample and silver nanoparticles**

**1.1 Green synthesis of silver nanoparticles -** Silver nitrate (AgNO3) was obtained from Pfizer pharmaceutical in India. Nutrient agar plates and nutrient broth (Difco, Becton, Dickinson and Company, Sparks Glencoe, MD, United States), along with potato-dextrose agar (PDA) plates, were sourced from a chemical shop in Agra. All clinical bacterial isolates were derived from the purification and isolation procedures conducted in the Microbiology lab at R.B.S. College, Agra. Antibiotic discs of streptomycin and gentamicin (5 mg/mL) were acquired from the chemical shop, while the plant extract disc was prepared within the Microbiology laboratory of R.B.S. College, Agra.

The bacterium E. coli was initially isolated in the laboratory in RBS college, Agra. Silver nanoparticles, averaging a particle size between 1-100 nm, were synthesized in the lab. For this purpose, 50 mL solutions of 1 mM silver nitrate were prepared in four different conical flasks. To each 50 mL of the silver nitrate solution, 1 mL, 2 mL, 3 mL, and 4 mL of plant seed extract were added, while maintaining the concentration of AgNO3 at 1 mM. Silver nanoparticles were synthesized at various concentrations of AgNO3, including 1 mM, 5 mM, 10 mM, and 15 mM, by adding plant extract in volumes of 1 mL, 2 mL, 3 mL, and 4 mL (Huang L, et.al., 2013). All these flasks containing silver nanoparticles were incubated in a dark cubicle to avoid the photo-activation of silver nitrate at 298K. The reduction reaction, where Ag+ is converted to Ag0, was confirmed by the transition of the solution's color from greenish to dark brown. Subsequently, the synthesis of silver nanoparticles (SNPs) was verified using UV–Visible spectroscopy (Kora AJ and Rastogi L.2013).

**1.2. Preparation and Characterization of Synergistic effect of Synthesized silver nanoparticles and Antibiotics:** A 1:1 ratio of antibiotic (128 µg/mL) to nanoparticles (128 µg/mL) was used to create an aqueous solution in order to examine the effects of Streptomycin and Gentamicin on the size, shape, and stability of the AgNPs. These solutions underwent characterization through SEM, TEM, DLS, zeta potential, UV-visible spectroscopy, and FTIR (McShan, D., et al., 2015). Additionally, to assess the chemical interaction between the AgNPs and antibiotics, we prepared an aqueous solution with higher concentrations of the antimicrobials (500 µg/mL), while maintaining the 1:1 ratio (Dana Khdr Sabir et.al.,2018).

**1.3. Checkerboard Synergy:** According to CLSI guidelines, the broth microdilution technique was used to determine the minimum inhibitory concentration (MIC) of each antimicrobial drug, whether it was used singly or in combination. In order to create different concentrations of 1,2,4, and 8 ml in the first row, a two-fold dilution of the antibiotic was added to each well of 96-well microtiter plates. In the first column, the AgNPs dilutions were similarly arranged, ranging from 0.75 to 0.125 µg/mL (Li, P., et. al., 2005). While the antibiotic dilutions were started from the first row down, the AgNPs dilutions were started from the columns on the right. As a result, different concentrations of the antibiotic and synthesised AgNPs were present in each well (Aabed K and Mohammed AE., 2021).

Each test microorganism was inoculated into the broth microdilution plates in 100 µL of Mueller-Hinton broth until the proper density (105 CFU/mL) was reached. The plates were then incubated for 24 hours at 370C, which is the ideal growth temperature. The lowest dilution that showed no turbidity was determined to be the MIC (Keshari A Kumar et.al.,2020).

**2 Analysis of Antibacterial Assay**

**2.1 Screening of Antimicrobial Activity -** By determining the lowest bactericidal and inhibitory concentrations of clinical pathogen antibiotics and synthesised AgNPs. In accordance with a standard procedure, the broth microdilution technique was used to assess the MIC of the biosynthesised silver NPs against clinical isolates (Faya AM et.al., 2010). At an initial concentration of 1 µg/ml, biosynthesised silver nanoparticles were serially diluted in sterile Muller Hinton Broth (MHB) (Oxoid). In order to achieve a final volume in 3 duplicates, 0.75 µl, 0.5 µl and 0.25 µl McFarland (108 CFU/ml of medium) of the bacterial suspension for each isolate was then added to each well. For twenty-four hours, microtiter plates were incubated at 37 °C. The smallest inhibitory concentration at which no discernible growth was seen was noted in order to look into the lowest concentration of NPs that prevent bacterial growth (Daima HK et.al.,2013). The broth microdilution method 25 was used to evaluate the clinical pathogens' minimum inhibitory concentration (MIC) to antibacterial drugs (Amanulla Mohammed Fayaz et. al.,2010).

**2.2 Determination of Minimum Inhibitory Concentration -** The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that visibly inhibits the growth of a microorganism after a specified incubation period. It is a critical parameter used to evaluate the efficacy of antimicrobial compounds, including antibiotics, plant extracts, synthetic drugs, and nanoparticle formulations. The MIC is commonly determined using broth dilution or agar dilution methods, with the broth microdilution method being the most widely employed due to its accuracy, reproducibility, and ability to test multiple concentrations simultaneously (S.Pal, et.al.,2010).

**2.3 Statistical Analysis -** Each assay was repeated three times. Data are presented as mean standard deviation (SD). The comparison between the effects of the two sources of variation was made using the two-way analysis of variance (ANOVA). The analysis was performed with the statistical software.

## III. RESULTS AND DISCUSSION

**1.Synthesis and Characterization of AgNPs:**

**1.1 UV-Vis Spectroscopy -** There are many methods for the Bio-synthesis of silver nano-particles but conjugated biosynthesis acquired more attention because of their synergistic effect with streptomycin. The UVspectroscopy shown that the conjugate prepared at concentration 1:1 ratio of plant extract and conjugate of silver nano-particles. Similarly, other concentration was taken as 1:2 and 1:4 ratio. The conjugates of silver nano-particles were prepared for Agonist effect of silver Nano-particles prepared with the help of Cyamopsis tetragonoloba plant.

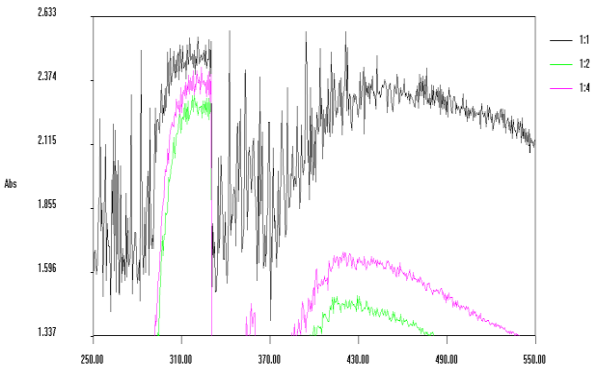



**Fig.1: UV-Visible Analysis of SNPs -Streptomycin conjugate**

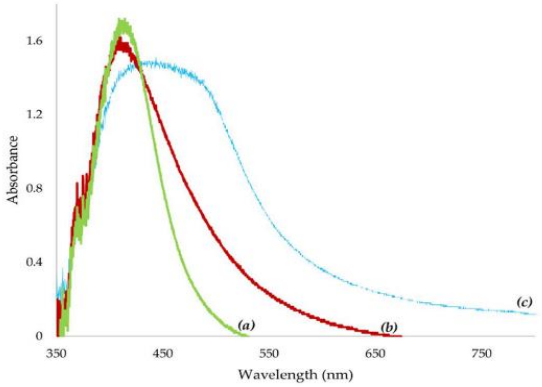
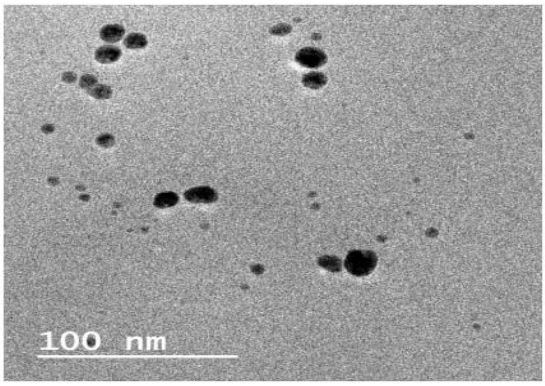
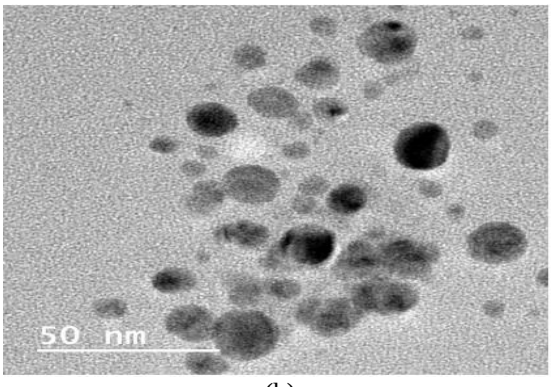



**Fig.2: UV-Spectroscopy showing Synergistic effects of synthesized silver nano-particles with Antibiotics (Streptomycin+Gentamycin)**

**1.2.TEM: Transmission Electron Microscopy (TEM)** is a high-resolution imaging technique that uses an electron beam, generated by electrically powered cathode rays, to pass through a thin sample and produce a magnified image. Similar to how light works in an optical microscope, the electron beam reveals fine structural details at the nanoscale. As shown in Figures 3(a) and (b), TEM effectively visualizes the synthesized silver nanoparticles, confirming their size at approximately 50 nm and 100nm, which indicates successful nanoparticle formation.




(a)




(b)

**Fig.3: TEM shows morphological dimensions of Silver Nano particles showing Particle range (a) 100 µm and (b) 50 µm**

**1.3. Scanning Electron Microscopy (SEM):** A small drop of the sample was placed on a clean glass slide and spread evenly to form a thin coating. Excess fluid was carefully removed using blotting paper, and the slide was then dried in a hot air oven for 15 minutes to ensure proper sample adhesion. To obtain a clear and high-resolution image under Scanning Electron Microscopy (SEM), the sample was coated with a thin layer of metal. In this investigation, silver was used for coating due to its excellent electrical conductivity, which helps reduce charging effects and enhances image clarity. The prepared slide was then analyzed using SEM, which provides detailed visualization of the sample's surface morphology. According to the SEM results, the particle size observed was up to 20 µm, as illustrated in Figure 4. This analysis confirms the surface structure and size range of the synthesized material.

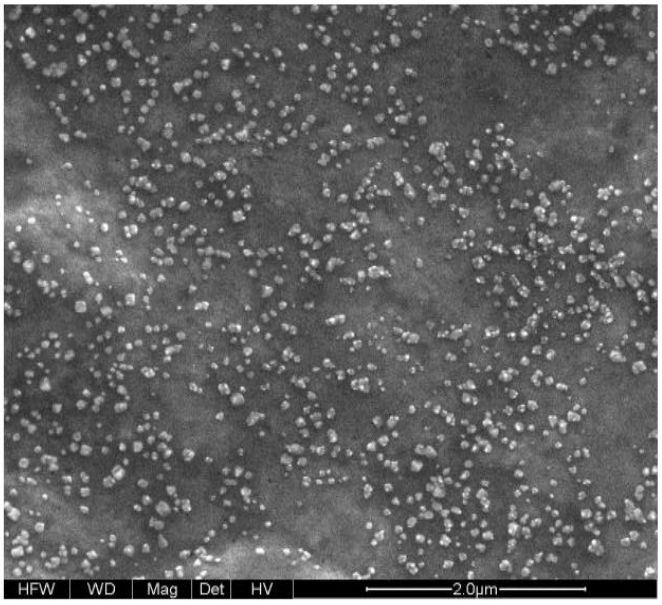



**Fig.4: The morphological evaluation shows dimensions of Silver Nano particles showing Particle range 20 µm**

**1.4. FTIR -** Few drops of sample were placed on to the sample occupant of FTIR-Spectroscopy and applied continues pressure and left for some time and recorded the Infra-Red absorbance wave number ranged from 4000 cm–1 to 400 cm–1 were observed and statistically analyses by using the 21 CFR part 11 software. Peaks were recorded separately for further study. The Infra-Red spectrum of silver nano particles shown bands at 3434.72 cm-1 and 3466.20 cm-1 shows phenol group stretch vibrational band phenolic. The peaks at 2832.15 cm-1 indicates C-H stretching of methlene group. The bands at 1630.98 cm-1 shows N-H group known as primary amines. The bands at 1363.07 cm-1 state C-H rock alkenes and bands at 1016.32cm-1 shows the availability of C-O group stretch and carboxyl group, esters and ether because all contain carbon and oxygen attached with double bond.

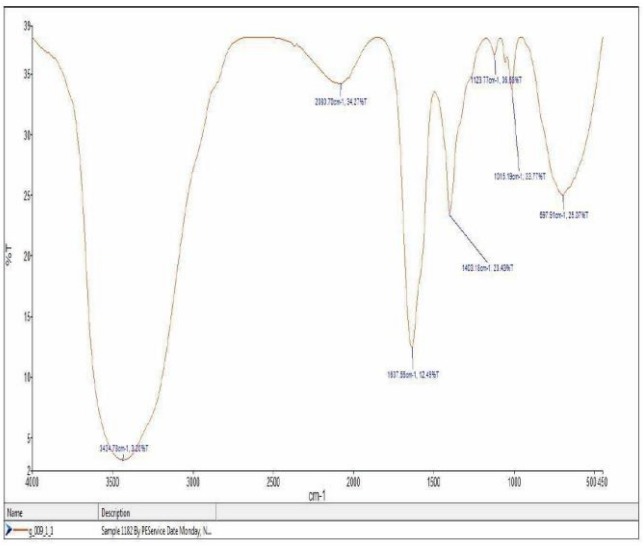
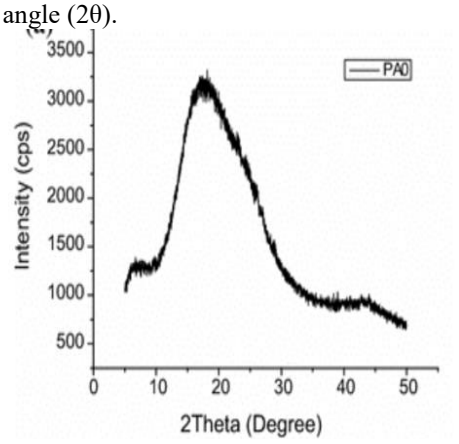


**Fig.5: FTIR showing stretching and bending of various Functional group**

**1.5 X-ray Diffraction (XRD)** - X-ray Diffraction (XRD) produces results in the form of a diffraction pattern, which is typically represented as a graph of intensity (counts) vs. diffraction angle (2θ).



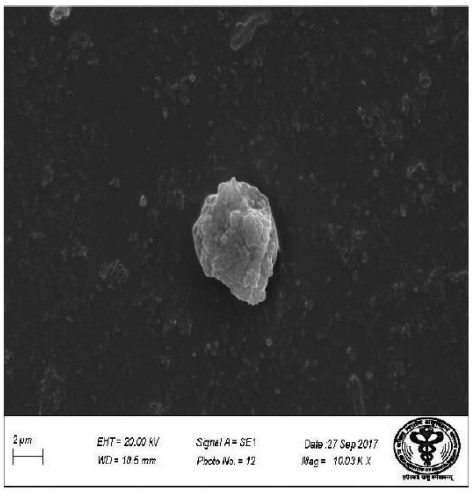




**Fig.6: (a)XRD Pattern (Intensity vs. 2θ Plot) (b) Showing size and amorphous shape of formed Silver Nanoparticle**

The X-axis represents the diffraction angle (2θ) in degrees. The Y-axis represents the intensity of diffracted X-rays (counts). Peaks in the pattern correspond to specific crystal planes within the sample.

**Peaks Indicate:** Peak Position (2θ): Indicates interplanar spacing (d-spacing), helping to identify the crystal structure. Peak Intensity: Related to the abundance of a particular phase in the sample. Peak Width: Broad peaks suggest small crystallite size or amorphous content, while sharp peaks indicate well-defined crystalline structures.

**1.6 Zeta potential** - The results of combination of synthesized AgNPs Gentamycin and Streptomycin showed a narrow distribution of sizes with a hydrodynamic diameter of 386.90 nm ±65.30 nm and a value of −21.10 mV ±4.63 mV for zeta potential. The increase of size of the nano particles are attributed to the addition of the streptomycin, which favours agglomeration of the AgNPs and cause an increase in the zeta potential.

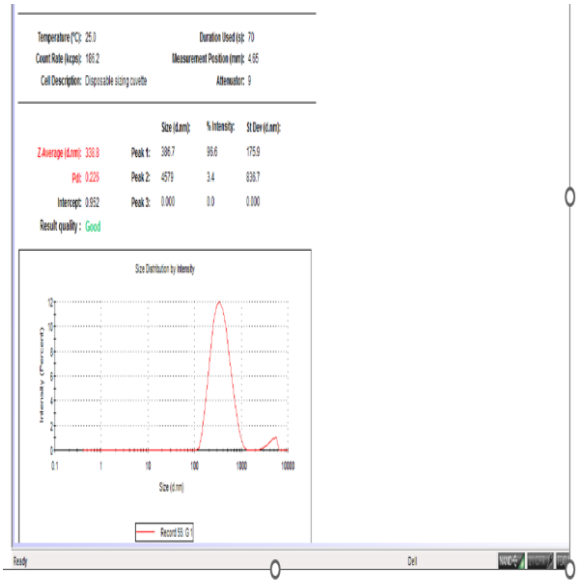


**Fig.7: Size distribution and intensity Graph of Zeta potential**

**2. Antimicrobial potential of silver nanoparticles against standard antibiotics.**

The synthesized silver nanoparticles are highly toxic towards pathogenic microbes. The antimicrobial activity of silver nanoparticles was analysed against two bacteria i.e. E.coli by using Disc diffusion method. The silver nanoparticles show good result and they are efficient against pathogenic microbes because of large surface area which give better contact with microorganism. In E.coli SNPs shows higher inhibitory zone. The bacterial outer membrane is made up of sulfur, SNPs interfere in this membrane and reaches to nuclear where it destroys the DNA which consist of phosphorus because phosphorus is sensitive to silver nanoparticle. So that DNA cannot be replicated and growth of microorganism is inhibited.

**2.1 Antimicrobial activity of control Streptomycin and Gentamycin**

| Antibiotic | Dose (mcg) | Zone of Inhibition (mm) against Pathogenic agent *E.coli* |
|---|---|---|
| Streptomycin | 10 | 36 |
| Gentamycin | 10 | 22 |

**Fig.8: MIC showing Minimum zone of inhibition of streptomycin & Gentamycin (control) in E.coli**

**2.2 Antimicrobial activity of synthesized silver nanoparticles against E.coli**

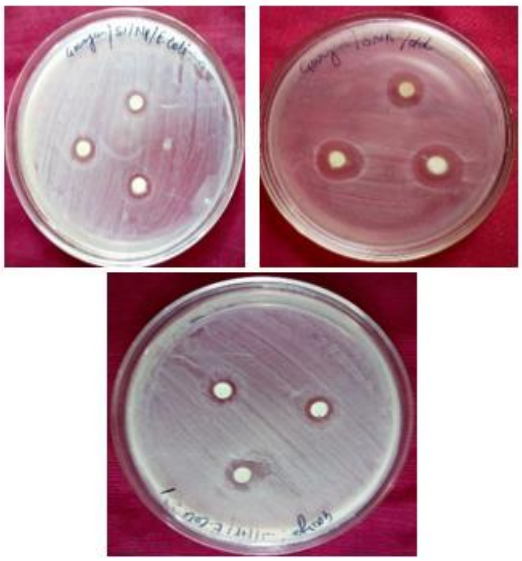


**Fig.9: MIC showing Minimum zone of inhibition in synthesized Silver Nano-particles against E.coli in various concentration in methanol solvent**

| S. No. | Solvent | Plant Part | Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubarion (*E.coli*) | | |
|---|---|---|---|---|---|
| | | | 0.75mg | 0.5mg | 0.25mg |
| 1 | Silver Nano particles | Seed | 15 | 12 | 10 |

A dilution was prepared to see antibacterial activity of silver nano particles and antibiotics like streptomycin and Gentamycin. The Muller-Hinton agar coated patridishes were prepared in which three were swabbed with E.coli bacterium. The dilution was prepared as 0.75µl SNPs solution was taken in Eppendrov's tube and 0.25µl of methanol added. In second eppendrov's tube 0.50µl SNPs solution was mixed with 0.50µl of methanol. Similarly, in third eppendrov's tube 0.25µl of silver nanoparticle solution added with 0.75µl of methanol. After that disc diffusion method was applied separately for E.coli. Then, patridishes were packed and kept in incubator at 370C for 24-48 hrs. The plates were examined for the zone of inhibition which is seen as clear area around the disc. It was found that E.coli zone is very effective. The approximate minimum inhibitory concentration obtained in E.coli is 15mm at 0.75µl concentration, at 0.50µl concentration is 12 mm while at 0.25µl concentration is 10mm.

## IV. STATISTICAL ANALYSIS

Analysis of variance or Anova is used for calculating the potential difference between the multiple group present in the study.

**Anova:**

Anova is statistical method used to compare various types of sample size at large scale. It included difference of means, mode and mediation amidst two or more group. This happens by seen at the multiple variation given in the data, so it was named as. Therefore, Anova is used for comparison and identifying the differences between the group and within the group. For example- in this study, two bacteria are identified and compared their minimum inhibitory zone at different plant extract concentration and against prepared Silver Nano-particles. After obtaining data this was analysed by Anova Statistical method.

**(iii)Satistical Analysis of data**.

| | | N | Mean | Std. Deviation | Std. Error |
|---|---|---|---|---|---|
| METHANOL *E. coli* | .25 | 12 | 23.08 | 4.981 | 1.438 |
| | .50 | 12 | 21.75 | 5.659 | 1.634 |
| | .75 | 12 | 24.58 | 3.801 | 1.097 |
| | Total | 36 | 23.14 | 4.876 | .813 |

ANOVA

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| METHANOL *E. coli* | Between Groups | 48.222 | 2 | 24.111 | 1.015 | .374 |
| | Within Groups | 784.083 | 33 | 23.760 | | |
| | Total | 832.306 | 35 | | | |

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 41.292 | 2 | 20.646 | .375 | .688 |
| Within Groups | 7756.458 | 141 | 55.010 | | |
| Total | 7797.750 | 143 | | | |

## V. CONCLUSION

Nano technology zestfully emerged as a paramount domain of Modern ideology with prospective effect in Microbiology and Biology. Nano biotechnology is mainly applied for the new investigation about the configuration and conglomeration of nanoparticles from metals used for diagnosis and treatment of disease with particle size less than 100nm in dimensions (Sayran Hamad Haji et. al., 2022). This coalition of silver nitrate with biological compounds or organic compounds tends to generate various diagnostic tools in many diseases like diabetes, obesity and important tool in cancer therapy. Nano biotechnology explains many applications in biological system for the production of new molecule in nano science. To create silver nanoparticles, an aqueous solution of silver nitrate (1 mM) was made and combined with a methanolic extract of Cyamopsis tetragonoloba seed extract under standard conditions. After 48 hours of being kept in a dark environment, this solution's hue changed from dark green to brown. This shift in hue verified that silver nanoparticles had formed. UV-visible spectroscopy was used to characterise the produced silver nanoparticles. The UV-Vis bands in the methanolic solution of seed extract had their greatest peak at 430 nm. Additional examination was conducted utilising TEM, SEM, and XRD (Gao, M., et. al., 2013) . It was discovered that the ideal particle size and shape ranged from 25 to 100 nm and had crystalline dimensions. FT-IR displays the current functional group's bending and stretching. Nano biotechnology combines biological structure and phytochemical to conglomerate Silver Nano-particles of accurate size which has particular obligation in different areas (Sondi.I et.al., 2004). It also represents a remunerative proxy over other method of formation of SNPs like physical and chemical method because it is green, eco-friendly, safe to handle for bio-synthesis of Nano-particles.

**Acknowledgement:** This research work was not funded by any Institution. This research work has been done in Raja Balwant Singh college, Agra (UP) and the technical part of the study completed in Indian Institute of Technology, Roorkee, (UP).
Conflicts of Interest: The authors declare no conflict of interest.

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