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Phytosynthesis, characterization and antimicrobial activity of silver Nano-particles by using *Cyamopsis tetragonoloba L.* plant seeds

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Abstract
Among several metals which are used for preparing nano particles, Silver metal is u tmost important and potential metal used to prepare silver Nano-particles. The silver Nano- particles were prepared by reducing method of silver nitrate in the presence of *Cymopsis tetragonoloba* seed extract. For the synthesis of silver nano particle aqueous solution of (1mM) silver nitrate is prepared and mix with the methanolic extract of *Cyamopsis tetragonoloba* seed extract under normal condition. Conglomeration of silver Nano particles were confirmed by colour change and PH change after 24 hrs. The integrated silver Nano particles were characterized using UV-Visible spectroscopy technique. Further characterization were done with the help of XRD, FT-IR, SEM,TEM, NMR and zeta potential. The average size of the formed silver Nano-particles were found to be between 1-100nm. Electromagnetic radiation absorbance was confirmed by NMR. Antibacterial activity of prepared silver Nano-particles done by using *Cyamopsis tetragonoloba* plant seeds extract and was studied against *E.coli* and *Bacillus subtilis* bacteria.

Keywords: *Bacillus Subtilis, Cyamopsis Tetragonoloba, E. Coli,* silver nano particles

1. Introduction
Cyamopsis tetragonoloba gum is a natural, soft colloidal and thickening agent used widely in food and industrial purpose. Its beans used for the cure of obesity, diabetes, for reducing level of cholesterol and treatment of diarrhea. This plant has great importance in medical industry, thatswhy, it is main centre of attraction for further research (Savita Chaurasia *et al.*, 2012) [12]. Nano Biotechnology is having great influence on human health and there approaches are growing enthusiasm in the medical sciences mainly in medicine, microbiology and bio nano-technology (Singh *et al.*, 2010 [11], Prabhu *et al.*, 2010) [10]. Many metals are available for the integration of Nano particles like copper, gold and Silver. Among all these metal NPs, Silver Nano particles are very encourage able and can be used in different fields like Microbiology, Biotechnology, Bio-medicine. Metallic Nano-particles ranges between 1 to 100mm have profound interest in studies.Genuinely, Silver is important for research because it does not create feasible environment for microbial resistant in to the host cell. Thatswhy, Silver metal is intermixed with materials like food stuffs, packing material and in fabrics. Phyto-synthesis of silver nano particles is better choice over other method used like synthetic, chemical method or micro-organisms methods because it is economically cheap, and environmental friendly. Medicinal plants have been used since ancient to cure many diseases because of their medicinal values. Therefore, Medicinal plants and their products are extensively studied in Chemistry, Bio-medicine, Ayurveda, Antimicrobial and essential oils (Bremner and Heinrich, 2002, Geetha *et al.*, 2002) [6]. Natural products like Lignin, tennin and Phenols obtained from medicinal plants used in Ayurvedic medicine as these medicinal plants are used in biological, biotechnological, microbiological and pharmacological activities. In current years, scientists and researchers are keenly curious in studying the wide applications of these medicinal plants by analyzing its vital ingredient liable for their remedial effects. Guargum (*Cyamopsis tetragonoloba L.*) is one of the vital medicinal plant which have lot of health benefits. *Cyamopsis tetragonoloba L.* has medicinal qualities because research on this plant proves that it is anticancerous, antimicrobial, antioxidant, anti-diabetic and very efficient in cure of cardiovascular disease and to control obesity.
The main issue of microbial resistance (MDR) is still extending continuously and the future prospects of antimicrobial medicines uses cannot be diverted. So, there is a need to take proper actions to reduce MDR related problem. The utilization of plant extracts with some additional molecules and phytochemical substances with familiar and good antimicrobial effects could have great importance in restorative treatments. The main objective of this study is to synthesized and characterized AgNPs by using the methanol extract of *Cyamopsis tetragonoloba* seed.

2. Materials and Methods
2.1 Preparation of Plant Extract
The Guar beans of *Cyamopsis tetragonoloba* was purchased from the market. The beans were then properly washed with the help of tap water and then washed with distilled water. After that Guar beans were dried under shade by covering with thin cloth to avoid dust particles. The dried beans were then crushed and grind with the help of Mortal and pastel. Then seeds were separated and homogenized to fine powder and stored in air tight container which were later used for solvent extraction.
Soxhlet Extraction Procedure: About 40 gm of *C. tetragonoloba* bean powder material were being uniformly

packed in to a thimble and put into Soxhlet extraction. It was depletable extracted in 200 ml Methanol solvent for the interval of approximately 48 hour or 22 cycles until the used methanol solvent in the siphon tube of an extract become colour less. After this the obtained seed extract was percolated through Whatman filter paper. Then obtained seed extract were kept in refrigerator at 4°C for further investigation of preparation of Silver Nano-particles and detection of antibacterial activity of prepared silver Nano particles and examine their physical and chemical properties

2.2- Synthesis of silver Nano Particles

1mM, 5mM and 10mM aqueous solution of Silver nitrate were prepared in Erlenmeyer flask. After that 1ml, 2ml, 3ml and 4ml seed extract were added to each 50ml of silver nitrate solution separately. All these Flask of silver nano particles synthesize was incubated in a dark cubicle to prevent photo-activation of silver nitrate at 298K. Reduction chemical reaction take place in which Ag^{+} to Ag^{0} oxidation state was confirmed by greenish colour of the solution changes into dark brown colour. After that, Synthesis of SNPs was confirmed by using UV–Visible spectroscopy.

2.3- Antibacterial activity of Silver Nano particles

Antibacterial activity is a procedure, in which killing of microorganisms and stops their development take place. The *in vitro* antibacterial testing of the silver seed extract and Silver nano-particles prepared from purified extract of *C. tetragonoloba* beans tested and established against the selected *E.coli* and *Bacillus subtilis* bacteria by using dilution method and minimum inhibitory concentration (MIC) methods. This method is used in a large amount of different samples to ascertain the amount of micro-organisms which are available in a specified population. This can be obtained by using the serial dilution method for bacterial population. This method provides statistically accurate information (Moteriya P. *et al.*, 2015) [9].

2.4 Characerization of Silver Nano particles

UV-visible spectroscopy is predominant technique used for detection of formation of silver Nano particles in different solvents. UV-Visible may be used qualitatively to classify functional categories, or to validate a compound's identification by matching the absorbance range.In Phyto synthesis, it is remarkable that phytochemicals present in the extract behave as reducing agent for integration of metal Nano particles. FTIR spectroscopy is an analytical technique used to consider the arrangement of specific molecules and the nature of molecular mixtures. Infrared light is absorbed by the functional groups found in the molecule at different frequencies similar to the vibrational bond energies. A characteristic band pattern is produced, this is the molecule's vibrational spectrum. In SEM a coordinated beat of high-vitality electrons is utilized by the filtering electron magnifying lens (SEM) to deliver a scope of signs at the strong examples surface. The signs getting from electron-cell communications show cell subtleties like outside morphology (surface), concoction creation, and crystalline structure and direction of test materials TEM used to analyze the development of semiconductor structures, their structure and defects. The consistency, form, thickness, and density of the quantum wells, cables, and dots can be examined with high precision. XRD (X-Ray powder diffraction) is a quick systematic strategy for the most part used to order a crystalline substance in a stage which may give subtleties on the unit cell estimations. The substance dissected is daintily secured, homogenized and characterizes the general creation of the stock. Crystalline materials characterization. Detection of fine-grained minerals such as mixed-layer clays and clays which are difficult to optically assess and determining the proportions of unit cells NMR is a method in analytical chemistry used in the quality assurance. This is used in science to assess a sample's composition and purity, as well as the most popular forms of nuclear magnetic resonance being carbon-13 and proton NMR spectroscopy, but that is true with any type of sample having nuclei with spin.its molecular structure. NMR spectroscopy to analyze chemical structures utilizing simple, one-dimensional techniques. The arrangement of more complex molecules is calculated using two-dimensional techniques.

3. Result and Discussion

3.1- UV-visible spectroscopy analysis

A surface plasma resonance spectrum of silver nano particle was procure at 398nm after 24hrs color change into green to brownish color. The graphical peaks represent the formation of silver nano-particles in the solution due to increase in surface plasma resonance. The Nano particles were scanned in the wavelength ranging from 250-550 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 45 nm and all aspects of peaks were detected. The value of peaks of the UV-Visible was recorded as in Fig-1.

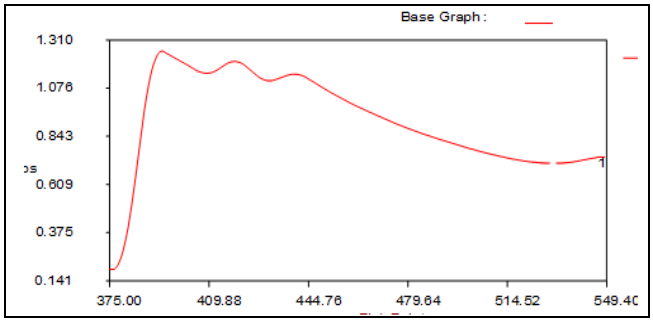

Fig 1: UV-Vis Spectroscopic Peaks and Pick points

3.2. FT-IR spectroscopy

The Infra-Red spectrum of silver nano particles shown bands at 3434.72 cm-1and 4000.20cm-1 (O-H stretching of phenolic bonds vibration). The peaks obtained at 2080.70 cm-1 indicates C-H bond stretching of methlene group. The bands present at 1637.66 cm-1 asserts primary amines. The bands at 1403.18 cm-1 state C-H asserts alkenes (alkyl group) and peak obtained at 1123.77cm-1 reveals the occupancy of C-O bonds alcohols stretching, 1015.19 cm-1 state carboxylic acids, 697.91 cm-1 states esters group and ether bonds. The reduction of silver metal to silver ions in the solution in this study is because of water soluble phytochemicals like flavanones, quinones, organic substances, other poly phenols, some proteins, metabolites like terpenoids which have functional group of alcohols, carbonyl group and carboxylic acid present in *Cyamopsis tetragonoloba* seeds appraised as the significant findings.

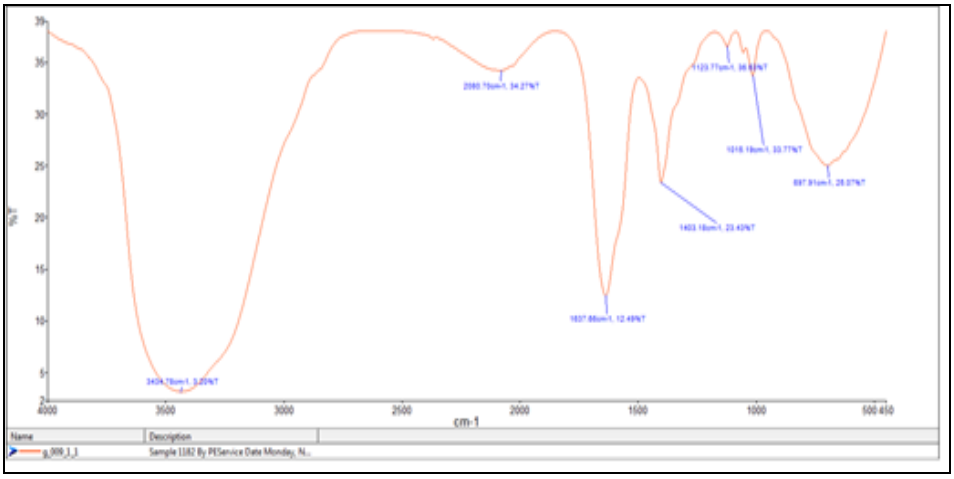

Fig 2: FT-IR showing various functional group stretching

3.3. Scanning Electron Microscopy (SEM)

A small drop of sample taken on slide then make a thin layer over glass slide, the residue were removed with the help of blotting paper then slide is kept in hot air oven to dry for 15 min. For getting clear image the sample need to be coating with metal. In this study, silver metal is used for coating because it is good conductor of electricity.SEM analysis determine that the particle range is between 2μm-5μm. The SEM result shown in below Fig-3

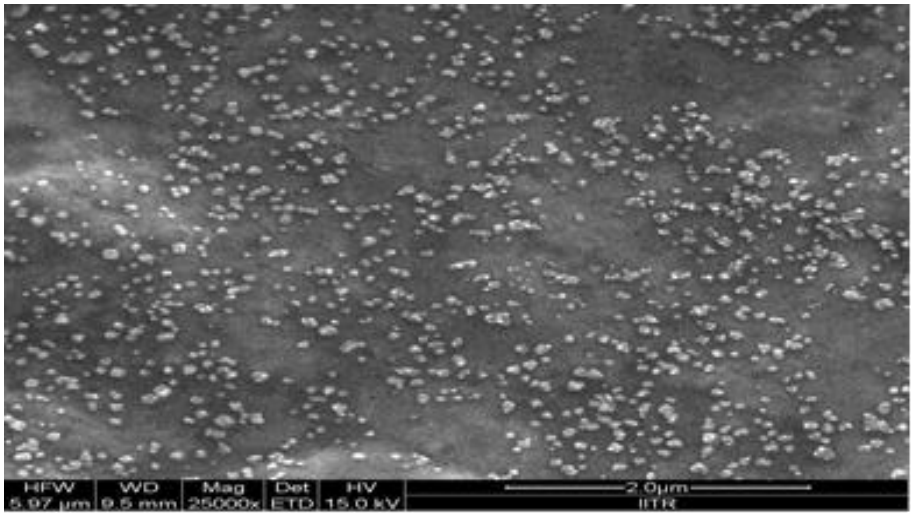

Fig 3: SEM, showing silver Nano-particle size in Between 2-5μm

3.4 Transmission Electron Microscope (TEM)

When thin layer of sample applied TEM generate a beam of electrons through the sample to produce a magnified image of the compound. The electricity provide the power to cathode rays as it develop a beam of electrons which works in alike to the beam of light works in an optical microscope to describe the particle size and shape as shown in Fig-4.

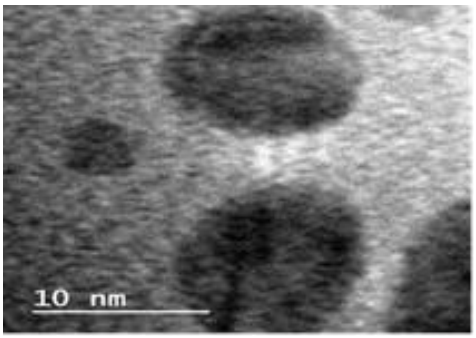

Fig 4: TEM, showing silver Nano-particle size 10nm

3.5- Nuclear Magnetic Resonance (NMR)

The synthesized structure of silver nano particle may look like a dendrimer which is because of functional groups present in the plant extract. The innermost proton of dendrimer contain encapsulated silver nano particles, so that ^{1}H-NMR signal resides close proximity to the silver nano particles. This may reduce or increase the receiving signals of NMR because of size of silver nano particles. The silver nano particles are assumed to be spherical in shape.

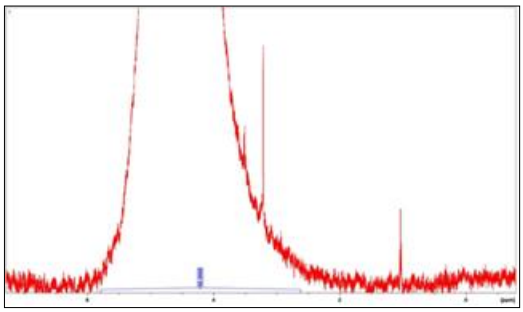

Fig 5: ^{1}H-NMR- spectrum of electromagnetic radiation

3.6-Zeta Potential

It shows the electrostatic repulsion forces between nearest oppositely charged particles in a dispersion medium. The small sized Silver Nano-particles showed high degree of zeta potential stability, the dispersion medium will counter aggregation.

If zeta potential has small electrostatic forces and this if this force may transcend and the dispersion medium breakout or agglomeration. Negatively or positively charged particles have high value of zeta potential and are electrically stabilized while those particles which have low value of zeta potential may be coagulate or agglomerate.

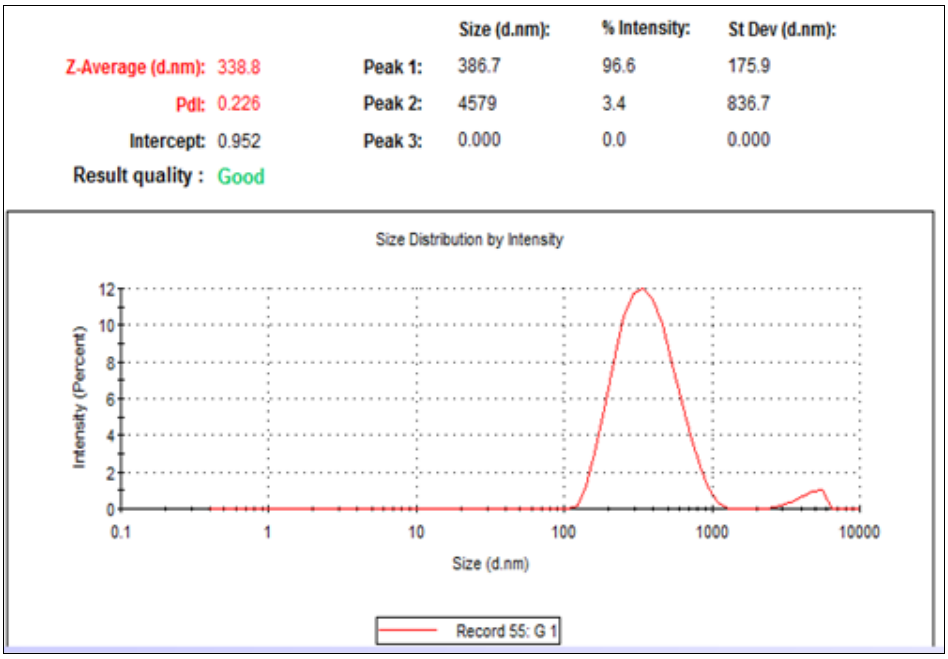

Fig 6: Size Distribuation v/s intensity graph of Zeta Potential

3.6-Antibacterial activity of Silver Nano particles-

Phytosynthesis of Silver Nanoparticles were done with the help of *C.tetragonoloba* seed extract which is considered as having high health benefits. Antimicrobial testing were done separately in Methanolic seed extract, Ethanolic seed extract and synthesized Silver nanoparticles in both Methanolic and Ethanolic seed extract against Gram +ve (*B.subtilis*) and gram-ve (*E.coli*) bacterium. Antibacterial activity of silver nanoparticles using Cyamopsis tetragonoloba seed extract shows effective minimum inhibitory zone against *E.coli* and *Bacillus subtilis* (Fig-7&8). Minimum inhibitory zone in *E.coli* is 10-15mm While in *Bacillus subtilis* is 9-12mm clearly mentioned in Table-1.

Table 1: Antimicrobial activity of silver nano-particles prepared with the help of *Cyamopsis tetragonoloba* against pathogenic microbe (*E.coli/B.subtilis)*

S. No.	Solvent	Plant Part	Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubarion *(E.coli/Bacillus subtilis)*		
			0.75mg	0.5mg	0.25mg
1	Silver Nano particles	Seed	**15/12**	**12/10**	**10/09**

Fig 7: Antibiogram of *silver nanoparticles in* Methanol seed Extract in *E.coli*

Fig 8: Antibiogram of silver nanoparticles in Methanol seed Extract in Bacillus subtilis

Discussion and Conclusion

The phytosynthesis of silver nano-particle is a low-cost, green, eco-friendly approach, capable to producing good minimum inhibitory zone against *E.coli* and *Bacillus subtilis.* These biologically synthesized silver nano-particles (*Cyamopsis tetragonoloba*) were found suitable for inhibitory effect on Obesity, diabetes and dyspepsia. Results clearly demonstrate the greater wound healing capacity of Guar-gum due to phenolic and other aromatic compound present in it. The optimum size of synthesized silver Nano-particles had great influence on the rate of chemical reaction for the reduction of compounds. In the investigation, it was found that the Tempreture influence the formation of size silver Nano-particle, as Tempreture increases rate of reaction will be slow down and size of the particle will be large.

The aqueous solution of silver nitrate was intermixed with *Cyamopsis tetragonoloba* seed extract, the confirmation of phytosynthesis of silver Nano-particles were observed by the colour change of plant extracts and further investigated by using UV-Vis spectroscopy. The organic compounds present in the seeds serve as an effective reducing agent.

These bio-synthesis silver nano-particles can potentially be used for different medical purpose like to diagnose disease and their treatment.

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