EFECT OF CHEMICAL AND GREEN SYNTHESISED SILVER NANOPATRTICLES ON UROPATHOGENS ISOLATED FROM UTI PATIENTS.

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INTRODUCTION:

Nanotechnology ("nanotech") is the manipulation of matter on an atomic, molecular and supra molecular scale and it is associated research and applications , ranging from extensions of conventional device physics to completely new approaches in all branches of science. Nanotechnology manipulates mater at the nanoscale (1-100nm) producing nano products and nanomaterials (NMs) that can have novel and size related physico chemical properties differing significantly from those from larger particles. The novel properties of NMs have been exploited widely in medicine cosmetics, environmental remediation Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material.

Nanoparticles often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example gold nanoparticles appear deep-red to black in solution. A nanoparticle of yellow gold and grey silicon is red in color. Gold nanoparticles melt at much lower temperatures (~300 °C for 2.5 nm sizes) than the gold slabs (1064 °C); Absorption of solar radiation is much higher in materials composed of nanoparticles than it is in thin films of continuous sheets of material. In both solar PV and solar thermal applications by controlling the size, shape, and material of the particles, it is possible to control solar absorption.

SILVER NANOPARTICLE:

Silver nanoparticles are nanoparticles of silver, i.e. silver particles of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures. Additional applications include molecular diagnostics and photonic devices, which take advantage of the novel optical properties of these nanomaterials. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria.

The widespread use of silver nanomaterials has the potential to adversely affect human and ecosystem health.

Due to the increasing prevalence of silver nanoparticles in consumer products, there is a large international effort underway to verify silver nanoparticles safety and to understand the mechanism of action for antimicrobial effects. Colloidal silver has been consumed for decades for its perceived health benefits but detailed studies on its effect on the environment have just begun. Initial studies have demonstrated that effects on cells and microbes are primarily due to a low level of silver ion release from the nanoparticles surface. The ion release rate is a function of the nanoparticles size (smaller particles have a faster release rate), the temperature (higher temperatures accelerate dissolution), and exposure to oxygen, sulfur, and light. In all studies to date, silver nanoparticles toxicity is much less than the equivalent mass loading of silver salts.

Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties.

- **Diagnostic Applications:** Silver nanoparticles are used in biosensors and numerous assays where the silver nanoparticles materials can be used as biological tags for quantitative detection.
- **Antibacterial Applications:** Silver nanoparticles are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their antibacterial properties.
- **Conductive Applications:** Silver nanoparticles are used in conductive inks and integrated into composites to enhance thermal and electrical conductivity.
- **Optical Applications:** Silver nanoparticles are used to efficiently harvest light and for enhanced optical spectroscopies including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS).

Silver nanoparticles have several characteristics that make it currently among the most widely used nanoparticles in science. One highly useful characteristic is its antimicrobial property. Silver in its pure form was known, even to the by ancient Greeks, as a great material to keep microbes at bay. If silver is transformed into a nanoparticle, this antimicrobial property is intensified, making it useful in effectively eliminating fungus, bacteria and viruses. As a natural material, silver is known to be safe to man and produce little to no allergic reactions when tested for curing various diseases.

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.Bar et al.,(2012) repoted that biogenic spherical silver nanoparticles were synthesized by a simple procedure using *Jatropha curcas* leaf extract as the reducing agent *Jatropha curcas* extract led to the formation of spherical silver nanoparticles of 15.2 nm ± 4.2 nm sizes.

Fang et al., (2008) biosynthesised silver nanoparticles using plant leaf extract in the size of 20–30 nm and it showed effective inhibitory activity against *Escherichia coli and Staphylococcus aureus*. Alteration in membrane permeability and respiration of the silver nanoparticles treated bacterial cells were evident from the activity of silver nanoparticles. Nature has devised ingenious and elegant way of creating the most efficient functional materials. In addition, the natural bioactive compounds like D-Glucose [Ravindran etal.,2006], glucan [Sen et al., 2013], sodium para-hydroxybenzoate tetrahydrate [Durai etal., 2014]; and saponins [Geethalakshmi and Sarada 2013] were previously used to synthesize Ag NPs. The reduction rate of metal ions is found to be much faster using bioactive compounds at ambient temperature and pressure conditions. The surface enhanced resonance properties of Ag NPs have been utilized for various biomedical applications as in sole or in combination with other smart materials [Sarkar et al.,2014]. The biomolecules and its mechanism on synthesis of nanoparticles still remain unexplored; understanding the mechanism will lead to discover the new paths in green chemistry. Specific characteristics of nanoparticles such as size, distribution and morphology which makes an impact in all the aspects of human life [Ashokkumar et al., 2014, Mariselvam 2014, Devanesan et al., 2018].

Memecylon umbellatum a medicinal plant was reported to possess various phytochemicals, among them polyphenols are considered as the most promising antioxidants. Polyphenols play many biological roles mainly attributed to their antioxidant activities towards scavenging free radicals, inhibiting peroxidation and chelating transition metals [Nicaver et al.,2012]. Phenolic compounds have the capacity to quench lipid peroxidation, prevent DNA oxidative damage and scavenge reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals [Cao et al.,2013]. The well documented anti-microbial activity of A NPs has led to the development of novel applications which made them as an alternative to antibiotics [Jeeva et al., 2014].

MATERIALS AND METHODS:

SYNTHESIS OF SILVER NANOPARTICLES:

Silver nanoparticles (Ag NPs) have wide range of applications due to their remarkable properties such as good conductivity, catalytic, optical properties and anti bacterial effect. There are many methods in which silver nanoparticles can be synthesized. They are

1. Green synthesis method
2. Chemical reduction method
3. Physical method
4. Mechanical method.

Among them, we chos e 2 methods which are economically significant. They are:

1. Green synthesis method
2. Chemical reduction method.

CHEMICAL REDUCTION METHOD:

The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such a sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions in aqueous or non-aqueous solutions.

Among the methods of synthesis of Ag NPs, chemical reduction method is the most commonly used one. The methods require the use of a reducing and stabilizing agent which in some cases can be the same. The nature and concentration of both reducing and stabilizing agents play an important role in size distribution and shape of NPs which greatly determines the NPs functional properties. This can also be controlled by careful tuning of other experimental conditions (e.g., temperature, rate of mixing, stirring, and time of reaction).

In this study the silver nanoparticles are synthesized by method:

1. Borohydride reduction method.

The chemicals used are:

- Silver Nitrate ($AgNO_3$)
- Sodium borohydride ($NaBH_4$)

Distilled water is used throughout the synthesis process. All the reactions are carried out at room temperature.

SYNTHESIS OF SILVER NANOPARTICLES

SYNTHESIS BY SODIUM BOROHYDRIDE REDUCTION:

The preparation procedure for synthesizing Ag NPs used for this study is explained with help of preparation procedure.

PREPARATION PROCEDURE:

Firstly a 100 ml solution of $AgNO_3$ (1mM) is prepared in a beaker. Then 10 ml of sodium borohydride (0.1M) is added drop wise to the beaker under continuous stirring, and stirring continuous for another 6 hrs. A change in the colour is observed and a solution of pale yellow is obtained. This mixture is allowed to rest for 8 hours and finally the yellow solution of AgNPs is stored at 4^oC before use. All the reactions are carries out at room temperature, using distilled water.

GREEN SYNTHESIS:

Silver nanoparticles (Ag NPs) prepared by green synthesis approaches that have advantages over conventional methods involving chemical agents associated with environmental toxicity. Green synthetic methods include mixed-valence polysaccharide, irradiation, and biological means. The green synthesis method was carried out in water, an environmentally-friendly solvent. Solutions of $AgNO_3$ containing glucose and starch in water gave starch-protected Ag NPs, which could integrate into medical applications. Ag

NPs synthesis by irradiation of Ag^+ ions does not involve a reducing agent. Eco-friendly biological mean like in plant contain proteins, which act as both reducing and capping agents forming stable and shape-controlled Ag NPs. Silver nanoparticles prepared by green synthesis method shows high antimicrobial activity against Gram-positive and Gram-negative bacteria. The mechanism of the Ag NP bactericidal activity is discussed in terms of Ag NP interaction with the cell membranes of bacteria.

Finally, environmental implications of Ag NPs to the ecology of aquatic environments are briefly discussed.

PREPARATION OF PLANT EXTRACT:

From *Bambusa vulgaris* (Bamboo) leaves:

Fresh leaves of, *Bambusa vulgaris* were collected from Anna University Chennai campus, and washed several times with water to remove the dust particles and then air dried to remove the residual moisture and ground to powder form. Then plant extract was prepared by mixing 1% of plant material with isoproponal (50ml) in a 250ml of (Borosil, India) conical flask. Then the solution was incubated for 2 days at room temperature. The supernatant was separated and filtered with (mm filter paper pore size) filter paper. Then the solution was used for the reduction of silver ions Ag (+) to silver nanoparticles (Ag -).

PREPARATION PROCEDURE:

Firstly a 10 ml solution of $AgNO_3$ (1mM) is prepared in a beaker. Then 5ml of Bamboo extract solution was prepared. Then 5 ml of $AgNO_3$ were mixed with the Bamboo extracts solution. The solution was kept undisturbed for 6 hours. A change in the colour of the solution was observed and the solution turned pale yellowish green colour is obtained.

GREEN SYNTHESIS:

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to the presence of some of the toxic chemical adsorbed on the surface that may have adverse effect in the medical applications. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route. So, in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advantage over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in there is no need to use high pressure, energy, temperature and toxic chemicals.

CHARACTERIZATION OF SILVER NANOPARTICLES

The characterization of nanoparticles is very essential to study the various properties of the material synthesized. The characterization provides information about the size, morphology, dimensions and the composition of the particle synthesized. Various techniques of characterization include:

.1 UV-VIS Spectrophotometer

.2 X- Ray Diffraction (XRD)

.3 Transmission Electron Microscopy (TEM)

.4 Energy Dispersive X- Ray Analysis (EDAX)

.5 Scanning Electron Microscopy (SEM)

.6 Particle Size Analyzer (PSA)

.7. Antibiogram of uropathogens

UV-VISIBLE SPECTROSCOPY:

The UV- visible spectroscopy refers to absorption spectroscopy in the UV- visible region. In this region of electromagnetic spectrum, molecules undergo electronic transition. The absorption spectroscopy measures transition from ground state to excited state. Samples are typically placed in a transparent cell, known as cuvette. It is typically a rectangular shape with internal width of 1cm made up of quartz crystal. The UV-visible spectrophotometer measures the intensity of UV-Visible light passing through the sample (I), and compares it to the intensity of light before it passes through the sample (I_o). The ratio of I/I_o is called the transmittance and is usually expressed as a percentage (%T). The absorbance A is calculated by $A=\log (I_o/I) = \log (100/T)$.

PROCEDURE:

The optical absorption spectra of Silver nanoparticle were recorded using a UV-Vis PG-T90. Initially base line adjustments are performed. The sample is taken in cuvettes with water as the solvent and placed in the setup and the absorption spectra is recorded in the wavelength range 200-900 nm

X-RAY DIFFRACTION:

About 95% of all solid materials can be described as crystalline. When X-rays interact with a crystalline substance (phase), one gets a diffraction pattern. In 1919 A.W. Hull gave a paper titled, “A New Methods of Chemical Analysis”. Here he pointed out that “every crystalline substance gipattern; the same substances always give the same pattern; and in a mixture of substances each produces its pattern independently of the others”. W.H. Bragg and William Lawrence Bragg developed a simple relation for scattering angles, now called as Bragg’s Law. Let us consider an X-ray beam incident on a pair of parallel planes, separated by an inter-planar spacing d. The two parallel incident rays 1 and 2 make an angle (θ) with these planes. A reflected beam of maximum intensity will result if the reflected waves represented by 1’ and 2’ are in phase. The differences in path length between 1 to 1’ and 2 to 2’ must then be an integral number of wavelengths, (λ). We can express this relationship mathematically as Bragg's law. Bragg's law is represented by the relation given by $n\lambda = 2d\sin\theta$

PROCEDURE:

The prepared samples were centrifuged and the precipitate was dried under vacuum and taken for XRD analysis. The XRD pattern was recorded using RigakuMiniflex IICTable Top XRD.

SCANNING ELECTRON MICROSCOPY:

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode.

Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons, photons, visible light, and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination). SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.

PROCEDURE:

Samples are dried in a glass plate with a drop placed in it and dried thoroughly and kept for sputtering under the gold plasma to prevent the charging of the particles present. Then samples are kept in the SEM chamber for the analysis. The images are obtained using Tescan Vega III SEM instrument.

PARTICLE SIZE ANALYZER:

Laser particle size analyzer works according to the optic properties, particularly to laser such as mono chromaticity, collimation, easily caused diffraction and scattering. In case beam illuminates the particles distributed in the liquid, the diffraction and scattering phenomenon take place. When the diffracted or scattered light passed through Fourier lens, a series of light rings emerge on the focal plane, radius of which are related to the size of the particles; the density of light is determined by number of particles. By circular array of photo detectors on focal plane, diffraction and scattering signals from the particles in different diameters are received. Then the signals are transferred to the computer through A/D conversion or other ways, being processed. The particle size distribution of the sample is thus obtained.

PROCEDURE:

The sample is taken in a cuvette and placed in the system. The refractive index of the material and the solvent is mentioned. And the system is initiated in the automated settings mode. The sample is scanned by a laser beam and the results are as shown below. The analysis were done using Malvern Zeta SizerNano series Nano-S instrument.

TRANSMISSION ELECTRON MICROSCOPE:

Transmission electron microscopy (**TEM**) is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through.

PROCEDURE:

Samples are dried in a glass plate with a drop placed in it and dried thoroughly and kept for sputtering under the gold plasma to prevent the charging of the particles present. Then samples are kept in the TEM chamber for the analysis. The images are obtained using TEM instrument.

ANTIBIOGRAM OF UROPATHOGENS

RESULT AND DISCUSSION:

UV VISIBLE SPECTROSCOPY:

UV- Visible spectroscopy was performed in spectrophotometer using distilled water as blank. The spectroscopy was performed for absorbance between the range of 200-900nm. For chemical synthesis peak was observed at 402nm and for green synthesis the peak was observed at 422nm.Silver nanoparticle shows the peak between the range of 390-430nm and this indicates the presence of silver nanoparticle in our sample.

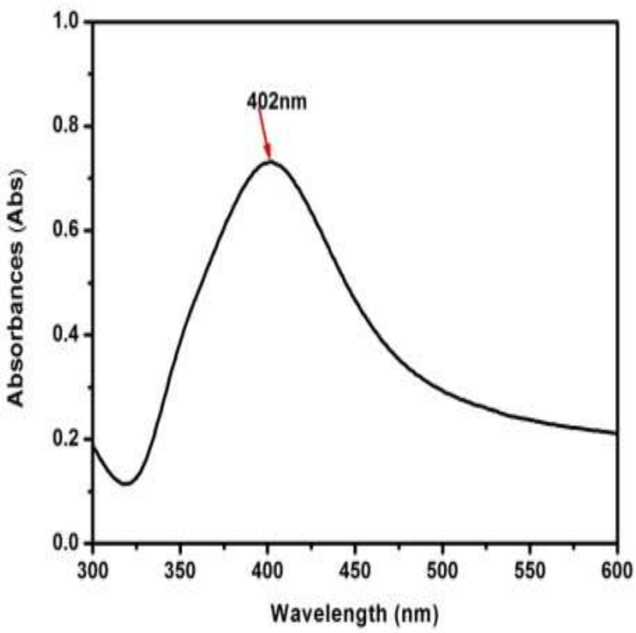

Fig: 1 UV- Vis spectrum of Ag nanoparticle (chemical reduction)

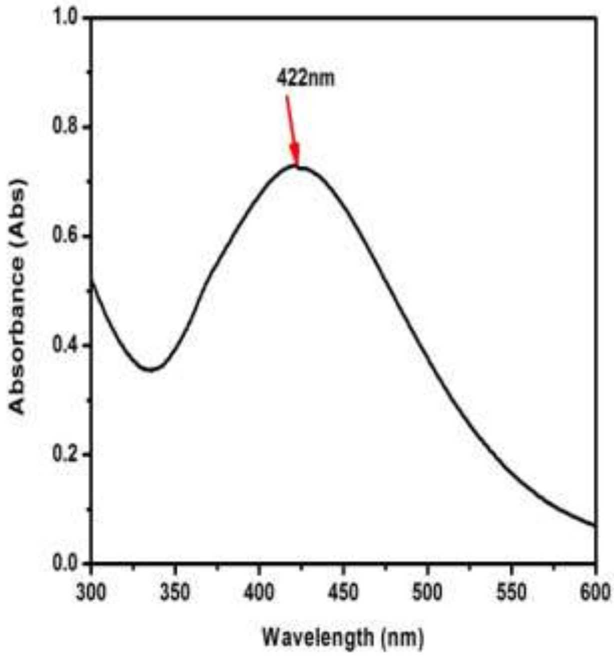

Fig .2 UV- Vis spectrum of Ag nanoparticle (Green synthesis)

X- RAY DIFFRACTION:

In the above graphs peaks are observed at 39.9°, 44°, 64.5°, 77.5° which corresponds to the {1,1,1},{2,0,0},{2,2,0} and {3,1,1} lattice planes. XRD measurement indicates that the remarkably intensive diffraction of the characteristic peak at 2ϴ value of 39.1 corresponds to the silver nano particles. The crystalline structure of AgNPs is face centered cubic (fcc) system with basal lattice plane (1, 1, 1). Additional peaks were observed and might be due to the shape modification of the nanoparticles which is further confirmed by SEM analysis. The broadness of the peaks indicates that the smaller size of the nanoparticle which is synthesized. The synthesized AgNPs does not have any crystalline impurities other than little unreduced to$AgNO_3$ and its byproducts.

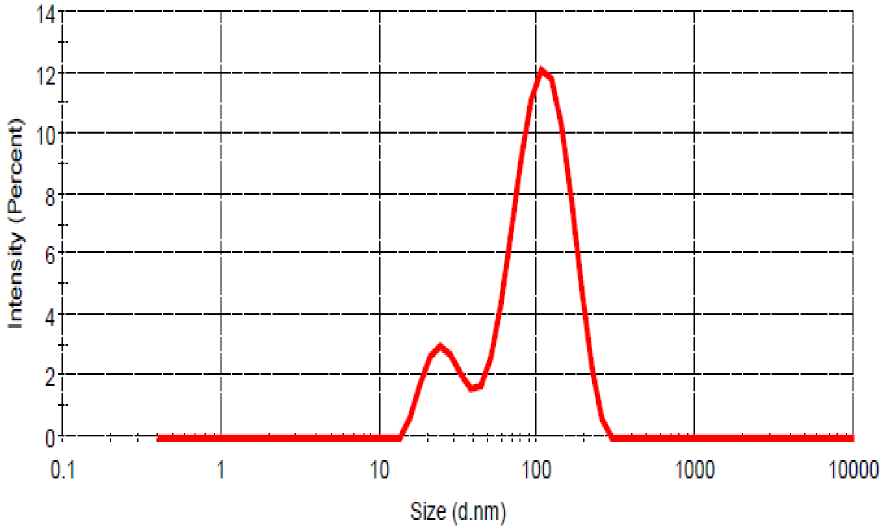

Fig 3:X-Ray diffraction patterns of AgNPs by Green synthesis

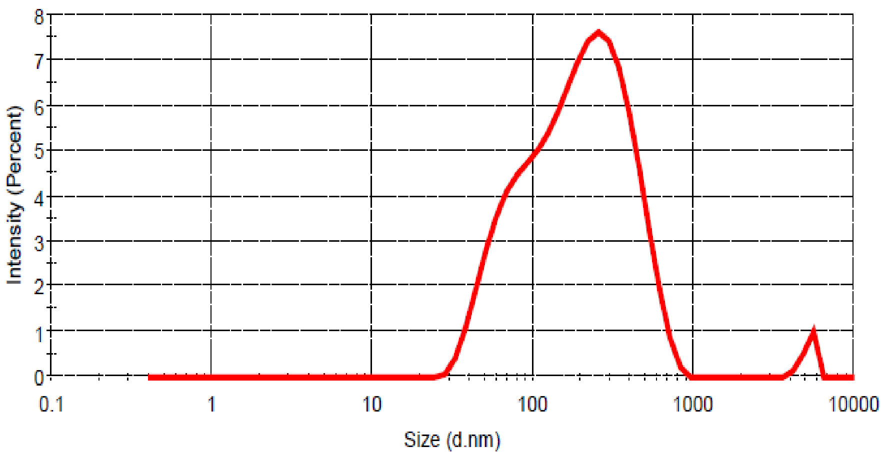

Fig 4 X-Ray diffraction patterns of AgNPs by chemical reduction method

SCANNING ELECTRON MICROSCOPE:

The particles synthesized by borohydride reduction are spherical in shape. They are smallest in size and are monodispersed in nature. Also we can see the agglomeration which is due to the surface charge of the nanoparticles. The nanoparticles are square shaped and are present in different size. This method has modified the shape of the AgNPs which has same size.

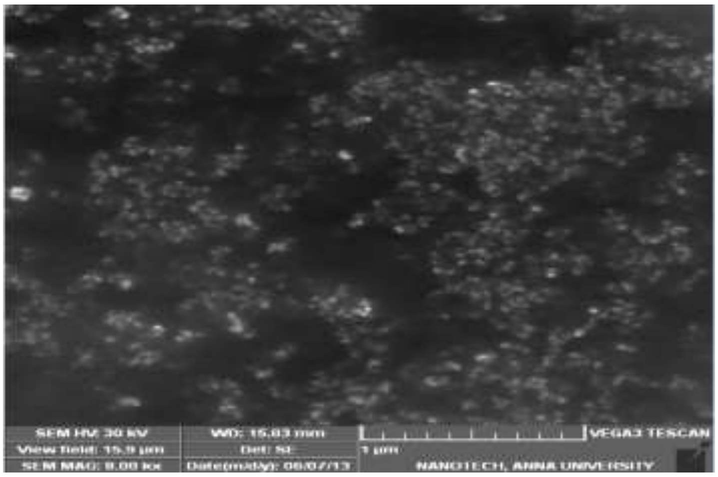

Fig 5: SEM images of AgNPs by chemical reduction method

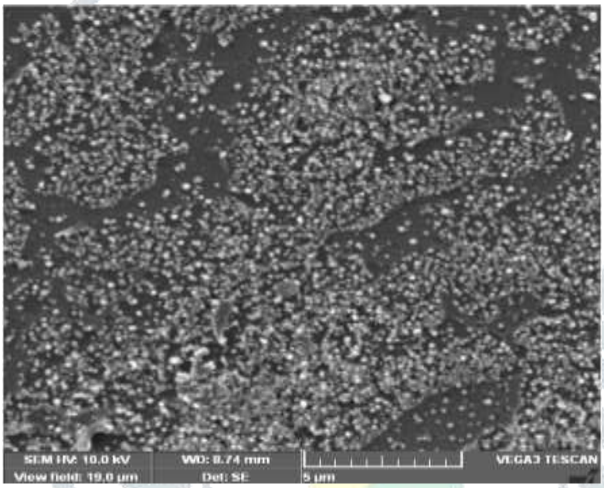

Fig 6: SEM images of AgNPs by synthesized by green synthesis method

TRANSMISSION ELECTRON MICROSCOPE:

The sample is fixed and viewed under transmission electron microscope to examine the internal view of the silvernanoparticles .The chemically synthesized nanoparticles were viewed under 0.1 and 0.2µm scales and spherically shaped nanoparticles were observed .The nanoparticles synthesized by green method were observed under 20nm and 50nm scales and the nanoparticles were found to be spherical in shape.

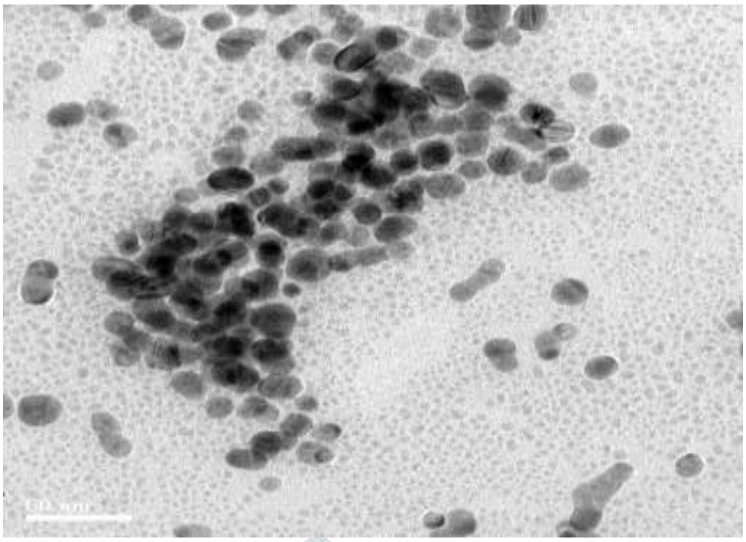

Fig 7 TEM images for chemical synthesised AgNPs

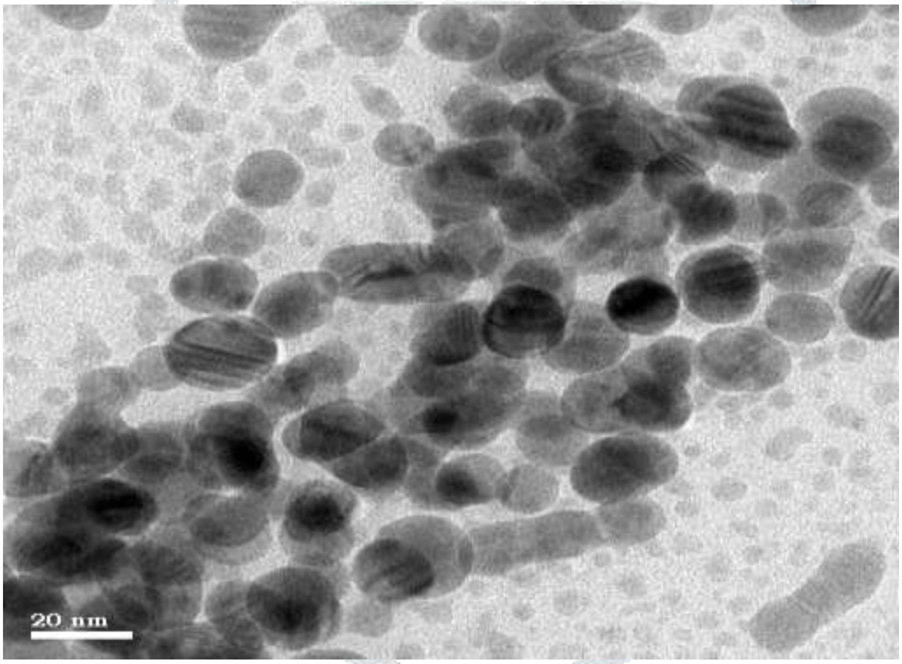

Fig: 8 TEM images for chemical synthesised AgNPs

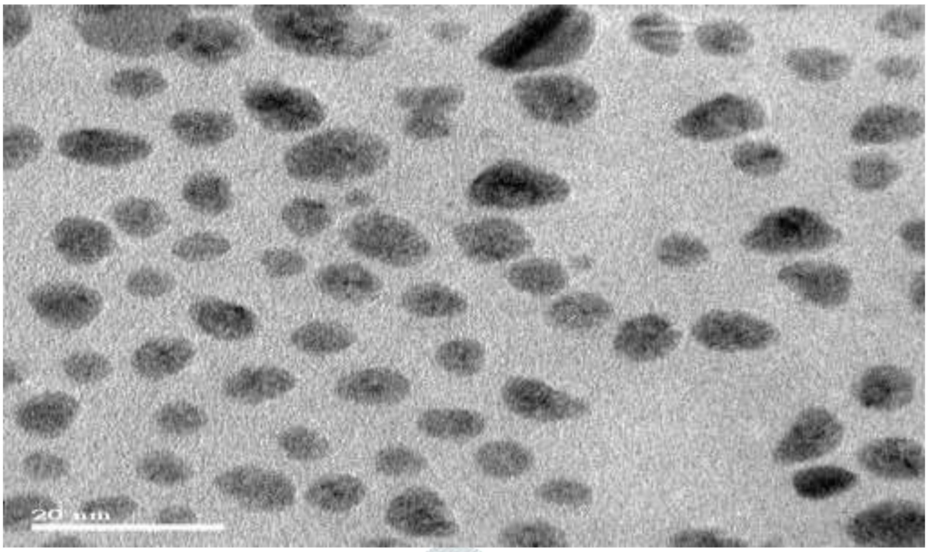

Fig: 9 TEM image for Green synthesised Green synthesized AgNPs

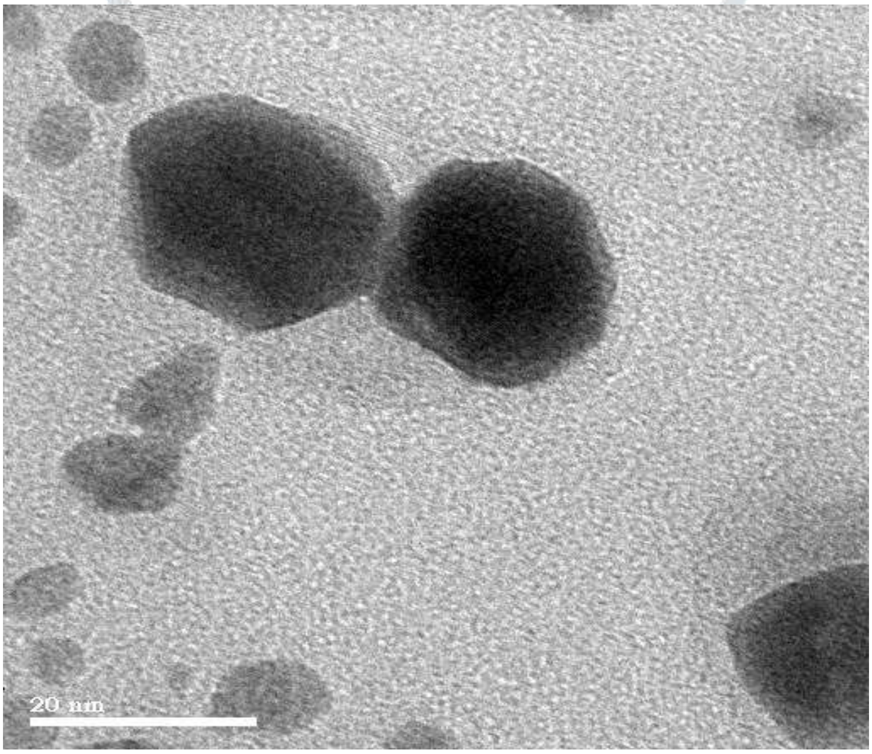

Fig: 10 TEM image of Green synthesized AgNPs

PARTICLE SIZE ANALYZER:

We observed the size of the nanoparticles by green synthesis method has the largest average size (57.54d.nm) while the Ag NPs synthesizes by the borohydride reduction method is smallest with average size (46.25d.nm). Apart from this all particles synthesized are monodispersed in nature and also we can able to see the agglomeration this is due to the surface charge of the nanoparticles.

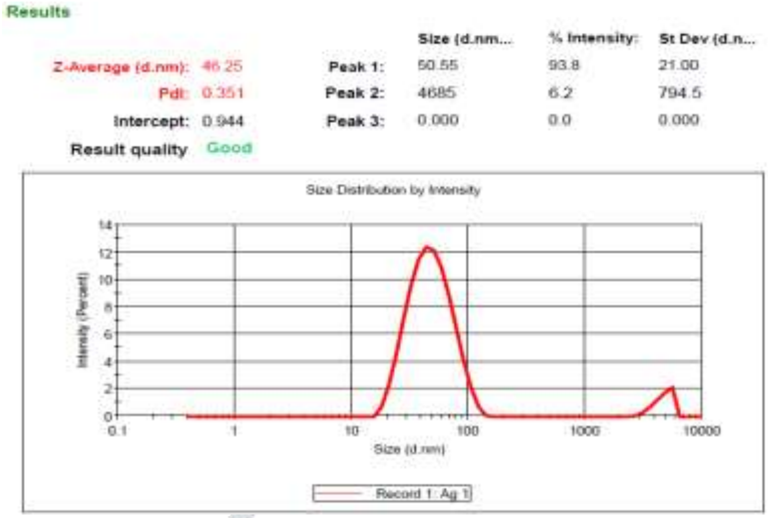

Fig 11 Particle size of AgNPs by borohydride reduction

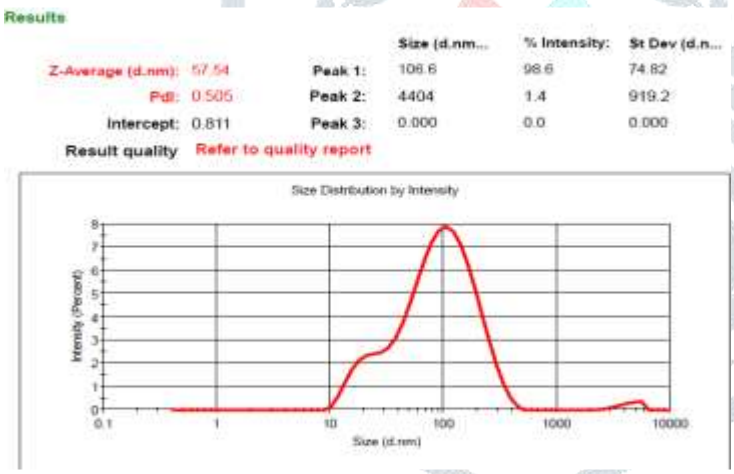

Fig 12 Particle of AgNPs by green synthesis method

APPLICATIONS OF SILVER NANOPARTICLES

ANTIMICROBIAL ACTIVITY ASSAY:

Among inorganic antibacterial agents, silver has been employed most extensively since ancient times to fight infections and control spoilage. The antibacterial and anti viral actions of silver, silver ion, and silver compounds have been thoroughly investigated .However, in minute concentrations, silver is nontoxic to human cells. The epidemiological history of silver has established its non toxicity in normal use. Catalytic oxidation by metallic silver and reaction with dissolved monovalent silver ions probably contribute to its bactericidal effect. Nature has devised ingenious and elegant way of creating the most efficient functional materials. In addition, the natural bioactive compounds like D-Glucose [14], glucan [15], sodium para-hydroxybenzoate tetrahydrate [16]; and saponins [17] were previously used to synthesize Ag NPs. The reduction rate of metal ions is found to be much faster using bioactive compounds at ambient temperature and pressure conditions. The surface enhanced resonance properties of Ag NPs have been utilized for various biomedical applications as in sole or in combination with other smart materials [18]. The biomolecules and its mechanism on synthesis of nanoparticles still remain unexplored; understanding the mechanism will lead to discover the new paths in green chemistry. Specific characteristics of nanoparticles such as size, distribution and morphology which makes an impact in all the aspects of human life [19].

Memecylon umbellatum a medicinal plant possesses various phytochemicals, among them polyphenols are considered as the most promising antioxidants. Polyphenols play many biological roles mainly attributed to their antioxidant activities towards scavenging free radicals, inhibiting peroxidation and chelating transition metals [21]. Phenolic compounds have the capacity to quench lipid peroxidation, prevent DNA oxidative damage and scavenge reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals [22]. The well documented anti-microbial activity of A NPs has led to the development of novel applications which made them as an alternative to antibiotics [23].

Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow-target antibiotics, because the metal attacks a broad range of targets in the organisms, this means that they would have to develop a host of mutations simultaneously. Thus, silver ions have been used as an antibacterial component in dental resin composites, in synthetic zeolites, and in coatings of medical devices.

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES:

The antimicrobial effects of silver (Ag) ion or salts are well known, but the effects of Ag nanoparticles on microorganisms and antimicrobial mechanism have not been revealed clearly. Ag nanoparticles were prepared and their shape and size distribution characterized by particle characterizer and transmission electron microscopic study. The antimicrobial activity of Ag nanoparticles was investigated against the UTI isolates *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae and Pseudomonas aeruginosa*. In these tests, Muller Hinton agar plates were used and Ag nanoparticles of various concentrations were supplemented in liquid systems. In the study it was found that the green synthesized AgNPs was more antibacterial than chemically synthesized AgNPs. *E. coli* exhiibited a maximum inhibition **in** 50 **µl** concentration in both type of AgNPs. nanoparticles,

Table 1. a-d.Table showing antibiotic resistance in UTI bacterial isolates against chemically synthesized and green synthesized AgNPs.

a.

Name of the organism	**Concentration of Ag nanoparticle(in µl)**	**Zone of inhibition (in mm)**
Escherichia coli	**10**	12 10
	20	13 11
	30	14 12
	40	15 13
	50	17 13

Name of the organism	**Concentration of Ag nanoparticle(in µl)**	**Zone of inhibition (in mm)**
Klebsiella pneumoniae	**10**	14 12
	20	15 12
	30	16 13
	40	17 14
	50	18 15

b

Name of the organism	*Concentration of Ag nanoparticle(in µl)*	*Zone of inhibition (in mm)*
Staphyllococcus saprophyticus	***10***	*13 11*
	20	*14 12*
	30	*17 12*
	40	*21 14*
	50	*21 15*

c .

Name of the organism	**Concentration of Ag nanoparticle(in µl)**	**Zone of inhibition (in mm)**
Pseudomonas aeruginosa	**10**	13 11
	20	15 12
	30	16 13
	40	17 14
	50	18 15

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