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BIOSYHTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM LEAF EXTRACT OF INDIGOFERA HIRSUTA. L. AND IT'S ANTIMICROBIAL ACTIVITY

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ABSTRACT

In present study silver nanoparticles were synthesized from aqueous leaf extract of *Indigofera hirsuta* L. plant leaf extracts will be collected were used and compared for their extracellular of metallic silver nanoparticles. Stable silver nanoparticles formed to be treated aqueous by solution of $AgNO_3$ with the plant leaf extracts as reducing agent Ag-to Ag^+. We successfully characterized the biologically synthesized Ag-nanoparticles, which had an average size of 18-20 nm. These nanoparticles showed antibacterial activity against *Pseudomoas aeruginosa* and *E. coli*. UV spectroscopy is used to monitor the quantitative formation of silver nanoparticles. The synthesized silver nanoparticles are characterized with FT-IR, XRD, SEM and EDX Characterization by the above said instrument analysis confirmed the presence, size and stability of the silver nanoparticles. After characterization, the silver nanoparticles were tested at various concentrations to check their bactericidal activity against clinical isolates of five bacterial pathogens. The silver nanoparticles exhibited good bactericidal activity at all concentration against all the tested organisms. Maximum zone of inhibition was of observed against *Vibrio cholera* (18mm at 400μg) and minimum level of antibacterial activity was observed against *Proteus mirabilis* (8mm at 100μg). This result suggested the potential use of silver nanoparticles against other clinical pathogens.

KEYWORDS: Indigofera hirsuta, silver nanoparticle synthesis,antibacterial activity against clinical pathogens.

INTRODUCTION

Nanoparticles is used to describe a particle with size in the range of 1nm100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for.

The enormous diversity of the nanoparticles arising from their wide chemical nature, shape and morphologies, the medium in which the particles are present, the state of dispersion of the particles and most importantly, the numerous possible surface modifications the nanoparticles can be subjected to make this an important active field of science now-a-days. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs. In recent year nanotechnology filed very important in the world. In 1974, Prof. Norio Taniguchi, was first to introduce the multidisciplinary. It is used for all research area discipline covering research and technology from physics, chemistry and biology commonly nanotechnology. The synthesis of nanoparticles has introduced nanotechnology during the last two decades that produced novel compounds applied in various fields.

Nanotechnology, involves design, synthesis, and manipulation of structures in particles with dimension smaller than 100 nm for specific functions nanoparticles are classified primarily into two types, viz organic and inorganic nanoparticles. The nanoparticles of carbon are called the organic nanoparticles. Silver (Ag) nanoparticles have high therapeutic potential and exhibit good antimicrobial activity.

Indigofera hirsuta L

Indigofera hirsuta (Fabaceae) is commonly known as hairy indigo has several medicinal uses. In Africa and in Kenya, it used as chest medicine and in Tanganyika whole plant is prepared as an external application for back ache (Burkill et al., 1935), nematicide, resistance to root-knot nematode, scorption bites ovarian and stomatch cancers (Djarwaningsih,1997).: whole plant extract is used in case of injury to the eye ball and inflammation of eye lids root decoction is used in most parts of Nigeria to counteract various poisons(Hutchin, son et al.,1958).

To study the preparation of metal nanoparticles in a simple, cost effective and eco-friendly way unlike chemical procedures, leaf extract of *Indigofera hirsuta* L. as a reducing and capping agent, determination of the size and shape of silver nanoparticles, characterization of silver nanoparticles by UV-Vis spectroscopy, FTIR, SEM,EDAX ,XRD analysis and *invitro* antibacterial activity of the synthesised *Indigofera hirsuta* plant leaf extract.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *Indigofera hirsuta.L* plant collected from Bharathidasan University campus at Trichy, Tamil Nadu, and India in the month of December 2015 and identified by the Rapinat Herbarium in St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Preparation of Plant Extract

The fresh leaves were washed with running tap water in 15 minutes and dried at room temperature for one week. Then the leaves are cut into small pieces and made into a fine powder.20g of leaf powder are weight and dissolved in 100ml distilled water in a 500 mL Erlenmeyer flask and boiled for 30 min. The extracts was filtered with glaze cloth again filtered with Whatman filter paper No.1, were stored in an airtight container and protected from sunlight until for further use.

Preparation of Silver Nanoparticles

1Mm of Silver nitrate ($AgNO_3$) was prepared in 1000ml bottle. The 100ml leave extract were mixed with 900ml silver nitrate solution in (1:9) ratio. Then kept in dark condition and colour change of the solution from reddish to brown indicated that the silver nanoparticles were synthesized. It is centrifuged in 7000 rpm at 28ºC for 15 min. Then collect the pellet and kept in hot air oven for dry and kept in O^0C until for further characterization studies and antimicrobial activity studies.

Characterization of Silver Nanoparticles

The characterization of silver nanoparticles was carried out by different instrument and technique. It includes visual observation, UV- Vis Spectrophotometer, FTIR, XRD, SEM, and EDAX.

UV- VIS Spectrophotometer

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300 to 600 nm using a UV–Vis spectrophotometer. The deionized water was used as the blank.

Fourier Transform Infrared Spectroscopy (FTIR)

In Fourier transform infrared (FTIR) analysis, the FTIR spectrum of the dried sample was recoeded on a PerkinElmer 1600 instrument in the range 400-4000cm-1at a resolution of 4 cm-1.The interaction between protein-AgNPs was analysed by Fourier transform-infrared spectroscopy (FTIR).

X-Ray diffraction analysis (XRD)

The X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles. The dried fine crystalline powder was need for Xrd analysis. The XRD spectrum confirmed the presences of silver nanoparticles. The diffracted intensities were recorded from 2Ө angle.

Scanning Electron Microscope (SEM) analysis

The silver nanoparticles were also characterized by scanning electron microscopy (SEM). The direct electron microscopic visualization allows measuring the sized and shaped of silver nanoparticles formed. SEM provided further insight into the morphology and size details of the silver nanoparticles.

Scanning electron microscopy (SEM) is a technique that uses electrons instead of light to form an output image. Since their development in the early 1950's, SEMs have thrown lights in many new areas of research including material science and nanotechnology. The SEM has allowed researchers to examine a much larger variety of specimens.

EDAX (Energy- Dispersive X-ray Spectroscopy) analysis

Energy-dispersive x-ray (EDX) spectroscopy analysis for the confirmation of elemental silver was carried out for the detection of elemental silver.

Antibacterial Assays

Antibacterial activity was assayed by well diffusion method. Totally five clinical isolates of bacterial pathogens namely *Escherichia coli, Salmonella typhi. vibrio cholera Pseudomonas aeruginosa, Pseudomonas mirabilis* were used for antibacterial assay. Nutrient agar medium was used for culturing bacteria. Chloramphenicol antibiotic was used as the positive control and sterile distilled water served as negative control. *Indigofera hirsuta* plant was collected and leaf extract different concentration of silver nanoparticles used for different assay .Fresh overnight culture were used for antibacterial activity.100µl of culture inoculum was spread using sterile swab onto nutrient agar plates. The plates were incubated at 37°C24 hours in dark condition and after incubation the zone of inhibition was measured

Silver nanoparticles

Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles (Klaus et al., 1999, Senaoati,S.,2005).

The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry).

Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Silver nanoparticles have a wide range of antimicrobial activities and exhibit high performance even at a very low concentration. Silver nanoparticles have been identified to possess good potential for the treatment of cancer (Govindan *et al.*, 2012). Nanoparticles play an important role in pharmaceutical, industrial and biotechnological applications. In particular, the silver nanoparticles are proved to have potential antibacterial, antifungal and anti plasmodial and larvicidal properties.

Nanotechnology involves tinkering work at atomic levels, tweaking and controlling substances 1, 00, 000 times smaller than a strand of human hair, to make useful materials and devices. It involves technology at the scale of one-billionth of a meter. The term 'NANO' is derived from Greek word "Dwarf" (Parthiban *et al.*, 2010). Nanotechnology is foreseen to significantly influence science, economy and everyday life in 21st century and it may become one of the driving forces to the next industrial revolution.

Silver has long been recognized as one of the nanoparticles having inhibitory effect on microbes present in medical and industrial process (Jose *et al.*, 2005; Lok *et al.*, 2007). The most important application of silver and silver nanoparticles is in medical industry such as typical ointments to prevent infection against burnt and open wounds (Ip *et al.*, 2006). Silver nanoparticles have diverse applications both in in-vitro and in-vivo (Haes and Duyne, 2002) conditions. Although there are many routes available for the synthesis of silver nanoparticles, biological synthesis using plant sources offers several advantages such as best in cost-effectiveness, non-toxic and eco-friendly agent (Aymonier *et al.*, 2002; Sun and Xia, 2002). It could be advantageous over other environmentally benign biological processes, as this eliminates the elaborate process of maintaining cell culture. Biosynthesis of nanoparticles by plant extracts is currently under exploitation.

This traditional synthesis method is more convenient for pharmaceuticals and biomedical applications (Goodsell, 2004). Biosynthetic processes of nanoparticles would be more useful, if nanoparticles are produced extracellularly using plants or their extracts in a controlled manner according to their size, shape and disparity (Kumar and Yadav, 2008). Although biosynthesis of gold and silver nanoparticles by the plants, Alfalfa (Gardea-Torresdey *et al.*, 2003), *Azadirachta indica* (Shankar *et al.*, 2004), *Emblica officianalis* (Ankamwar *et al.*, 2005).

RESULT AND DISCUSSION

Leaf is used against infant immunity (Suvarnalatha et al., 2004) urinary complaints (Gaue et al 2010) decoction of leaf is used in case of stomach problems (Faridah Hanum and Vander means et al 2007) used against diarrhoea (Siva et al2007) *I. hirsuta* leaf methanol and ethanol extracts showed effective activity at 100µg/ml of *E.coli* and *B.Subtilis* than *P.aeruginosa* and *S.aureus*, which also supports the presence of flavonoids like rutin, quercetin; kaempferol, luteolin, apigenin, orientin and phenols like protocatecheuic acid, chlorogenic acid, trans-p-coumaric acid, cis-p-coumari acid, p-hydroxy benzoic acid, coumarin and cinnamic acid. Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm in one dimension. Remarkable growth in this up-and-coming technology has opened novel fundamental and applied frontiers, including the synthesis of nano scale materials and exploration or utilization of their exotic physicochemical and optoelectronic properties.

Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, reorography, single electron transistors, light emitters, nonlinear optical devices, and photo electrochemical applications (Colvin, V.L.S.M.C& Alivisator,A1994, Wang Y. ,H.N.,1999).To achieve this kind of synthesis, there is intense need of green environment benign processes, which happen to be mostly of a biological nature. Biosynthesis of silver nanoparticles (AgNPs) offers a valuable contribution as eco-friendly technologies of materials science. In addition to the ability of some microorganisms such as bacteria (Shahverdi et. al., 2007) and fungi (Vigeshwaran et. al., 2011 & Salunkhe et. Al., 2011), recently, the exploitation of plant resources has materialized as a novel method for the synthesis of silver nanoparticles.

Silver nanoparticles have been synthesized using various natural plant products from Azadirachta indica (Teipathy et. al., 2009). Many species belonging to Euphorbia genera have milky latex, which exudes copiously when cut except Jatropa sp., which produce clear sap. Latex especially from the genus Euphorbia is chemically defined by the occurrence of a large number of poly functional diterpenoids with the tigliane (phorbol), ingenane, and daphnane skeletons (Evans & Soper 1978); lectins; and lysozymes with potent biological activities (Wititsuwannaku, et. al., 1998; Keefe 2001).The members of this family are reported for their medicinal properties in ethnobotany (Kubmarawa et. al., 2007). Pedilan thustithy maloides has been studied for its antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli (Vidotti et. Al., 2006).

Mandal et al., (2006) reported that in the last decade, the utilization of biological systems has emerged as a novel and reliable method for the synthesis of nanoparticles due to a growing need to develop eco- friendly process in nanomaterials synthesis.

Silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively. The silver nanoparticle act on a broad range of target sites both extracellularly as well as intracellularly. In fact microbes generally have a harder time in developing resistance to silver than then they do to antibiotics (Jain et al., 2009).Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver Nanoparticles in medical industry such as tropical ointments to prevent infection against burn and open wounds. The nanoparticles exhibited antimicrobial activity against *E.coli* and *Pseudomonas aeruginosa* as it showed a clean inhibition zone, whereas the standard antibiottics like Ampicillin, Tetracycllin, Cefixime and Rifampicin does not show any zone. Antimicrobial effects of AgNP's obeyed dual action mechanism of antimicrobial activity, i.e, the bacterial effect of AgNp's and membrane-disrupting effect of polymer subunits. (Jain et al., 2009)

Antibiotics have been used as therapeutic and prophylactic treatments to control a variety of bacterial infections in livestock for more than 50 years. Different types of antibiotics have also been fed at sub-therapeutic levels to cattle, poultry and swine to increase productivity and feed efficiency (McDermott et al., 2002).Lakshmi s.nair et al., (2007) reported the silver nanoparticle synthesis and therapeutic applications. The unique optical, electrical and biological properties of metallic nanoparticles have attracted significant attention due to their potential use in a host of applications such as catalysis, bio sensing, drug delivery and Nano device fabrication. M. Rai et al., (2009) reported that Nano crystalline silver particles have found tremendous applications in the field of high density bimolecular detection diagnostics and antimicrobials.

Sukumaran prabhu et al., (2012) reported the silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Kantha *et al*.,2013 reported the synthesized silver and gold nanoparticles were characterized by ultraviolet (UV)-visible spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analysis. Kantha Deivi *et al*., 2014 reported the UV- visible spectrum of the bioreduced GNP shoed maximum peak at 536 nm and, structural studies by Scanning Electron microscopy showed the triangular structure of the GNPs. The elemental composition was confirmed by EDAX. The mono-crystalline nature and the of the green synthesized Aeglemarmelos GNPs were confirmed by XRD analysis and the Bragg reflections obtained from the gold nanotriangle clearly correspond to the face center crystalline structure of the gold. The FTIR spectroscopic study has confirmed that the carbonyl group of amino acid residue and peptides of proteins of the plant extract has strong ability to bind metal, and most possibly might have formed a layer on the gold nanoparticles.

Jyoti Ahlwat *et al*., 2015 isolated the plant Interest in "green nanotechnology" in nanoparticle biosynthesis is growing among researchers. Nanotechnologies, due to their physicochemical and biological properties, have applications in diverse fields, including drug delivery, sensors, optoelectronics, and magnetic devices. This review focuses on the green synthesis of silver nanoparticles (AgNPs) using plant sources. Green synthesis of nanoparticles is an eco-friendly approach, which should be further explored for the potential of different plants to synthesize nanoparticles. The sizes of AgNPs are in the range of 1 to 100 nm. Min chung *et al.*, 2016 studied the Silver nanoparticles were confirmed by colour changes. UV-Vis spectrophotometer showed broad peak located at 452 nm. The TEM images showed formation of circular, triangular, rectangular and oval shapes AgNPs in the size of 1.5-25 nm. Smaller size makes silver nanoparticles a better bactericidal. FTIR peaks were from 375-4000 cm-1 which confirmed the formation and stabilization of silver nanoparticles. So findings from this study suggests a better alternative for simple, rapid and eco-friendly synthesis of green AgNPs from leaf extract of *Capparis decidua L.* The synthesized AgNPs could be used in various biomedical, optical and electronic applications,

Characterization of silver nanoparticles

UV-Vis Spectroscopy

Primary conformation of the AgNPs was carried out by UV- Visible spectrophotometric analysis. The nanoparticles show maximum absorbance beak at 420nm on UV - Vis spectra which is shown in Fig 1.

FTIR Analysis

FTIR spectrum was analysed for identification of different biomolecules adsorbed on the surface of nanoparticles, and also to find out their role in reduction and stabilizing the nanoparticles The FTIR spectrum of synthesized silver nanoparticles by the *Indigofera hirsuta* leave extract, figure 13shows strong bands at 3436.3,2927.6,1573.4,1384.8,1116.5 and 1116.5cm-13436.3cm-1 corresponds to and phenols O.H groups alcohols, phenols .A peak at 1573.4cm-1 corresponds to secondary amine. The spectral bands (1450-1600 cm-1) shows presence of proteins which are responsible for the reduction of metal ions or affinity for metal nanoparticles. The bands (1300-1450 cm-1) suggest the presence of flavanones/terpenoids adsorbed on the surface which are very abundant in *Indigoferaa hirsuta* plant, while nanoparticles bond showed strong peak at1116.5cm-1.

From the analysis of the FTIR spectrum, carboxyl group were found adsorbed on the particles surface, hence that confirms the presence of biomolecules like terpenoids, flavonoids which acts as a capping agent for the synthesized nanoparticles. This also throw some light on the dual role of biological molecule in reducing metal ions and capping .Capping of nanoparticles by protein stabilizes silver nanoparticles and prevents agglomeration in the medium. FTIR analysis confirmed that the *Indigofera hirsuta* leaves extract can perform dual functions of reduction of (Ag+) to (AgO) and also stabilization of silver nanoparticles.

Sscanning Electron Microscopy

Scanning Electron Microscopy is done for revealing the surface morphology of particles. Here, the bead for the SEM analysis was prepared by placing a drop of the silver nanoparticle suspension on the carbon tape attached to the head of cylindrical bead and it was dried inside a vacuum dryer for a couple of hours. The particles on the top of the bead were scanned by Scanning Electron Microscope and the following image (Fig.3) was obtained.

Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy or EDX is a technique that is mainly used to identify the presence of different elements in a sample. It is necessary to verify the presence of desired element in a sample. In the present study, this technique was used to verify the presence of Ag and the curve (Fig 24) showed a small peak of the element along with those of C and O.

In the present study, for the conformation of AgNPs, EDAX spectroscopy analysis was performed, which confirmed the presence of elemental silver by the sharp signals (Fig.4) at 3Kev

X-Ray Diffraction

Fig .5 shows that the XRD Pattern of vacuum dried silver nanoparticles synthesized using latex of *J.curcas*. A number of brag reflections with 2θ Values of 38.03°, 46.18°, 63.43°, and 77.18° sets of lattice plans are observed which may be indexed to the (111),(200),(220)and (311) facets of silver respectively. XRD Pattern thus clearly illustrates that the silver nanoparticles formed in this present synthesis are crystalline in nature.

Antibacterial activity

In the present study, the assay for antibacterial activity was done against *E.coli*, *Salmonella typhi*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Proteus mirabilis* with various concentrations (100,200,300 and 400µg) of green synthesized silver nanoparticles of *Indigofera hirsuta* and resulta are shown in fig.6. The silver nanoparticles showed good activity against all tested organisms at all concentrations. The highest zone of inhibition was found against *Vibrio cholera (*18mm at 400μg) and minimum level of antibacterial activity was observed against *Proteus mirabilis* (8mm at 100μg). The above observation clearly indicated that the synthesized silver nanoparticles have the potential to kill the bacteria effectively.

Pongamia pinnata leaf solvent extract was subjected to synthesis of silver nanoparticles and the visible colour change indicates the formation of nanoparticles which was confirmed by UV-visible absorption spectroscopy. The progress of the reaction between metal ions and the leaf extracts were monitored by UV-visible spectra of silver nanoparticles in aqueous solution with different reaction times (Bannoth Reddya Naik *et al*., 2014) reported that isolated in general UV-Vis spectroscopy was a valuable tool to examine size and shape controlled nanoparticles in aqueous suspensions. The Ag NPs production was monitored with the colour change through visual observation and UV-Vis spectroscopy.

FTIR analysis was carried out to identify the possible biomolecules present in the Leaves. Prominent IR bands are observed at 674, 962, 1227, 1381, 1451, 1531, 1644, 1786, 2308 and 2353 cm-1. Most of the IR bands are characteristic of flavonoids, phenols, alkenes and carboxylate groups present in the synthesised nanoparticles. The peak at 674 cm-1 attributes to C-Cl stretching modes of alkyl halides. A strong band at 960 cm-1 corresponds to the stretching vibrations of C-O-CH3 and -OH bending of carboxylic acids (Gunasekaran and Ponnusamy, 2005). Another strong band at 1644 cm-1 is recognized, related to the carbonyl stretching vibration in the amide-I linkages of the proteins (Narayanan and Sakthivel, 2011).

A SEM employed to analyze the morphology and size details of the silver nanoparticles that were formed. It was showed that the silver nanoparticles formed were spherical in shape, with the size ranging from 30nm to 110nm and uniformly distributed silver nanoparticles on the surface of the cells were observed. A similar phenomenon has been reported (Chandran *et al.*, 2006,Saraniyadevi *et al* ., 2012)

Elemental composition information of Ag NPs was carried by EDS analysis .EDS spectrum confirms the presence of strong elemental signal of the silver approximately at 3 keV which is typical for the absorption of metallic silver nano crystallites due to surface plasmon resonance (Kalimuthu *et al*., 2008)

The Ag NPs synthesized using *A. precatorius* leaf exerted a fairly significant antibacterial action on the tested bacteria. the zones of inhibition of Pseudomonas spp. and E. coli. Pseudomonas spp. depicted the highest sensitivity to nanoparticles compared to other organisms and was more adversely affected by Ag NPs that were observed from the inhibition zone in disc diffusion method. Next to this, Staphylococcus spp. and Bacillus spp. were inhibited by Ag NPs. A very small but noticeable zone of inhibition was observed for E. coli. This symbolizes the potentiality of Ag NPs as an antibacterial agent. It is evident by the values of diameter of zone of inhibition obtained during assessment of antibacterial activity (Ajitha et al 2014)

FTIR analysis was used further for the characterization of the resulting nanoparticles. FTIR absorption spectra of silver nanoparticles showed absorbance bands (before bioreduction) in the region of 3436.3, 2927.6, 1573.4, 1384.8, In particular, the 1116.5cm-1 band arose most probably from the O.H groups alcohols, phenols such as hydroxyl flavones and catechins. The total disappearance of this band after the bioreduction might be due to the fact that the polyols were mainly responsible for the reduction of silver ions, whereby they themselves got oxidized to unsaturated carbonyl groups leading to a broad peak at 1116.5cm-1 for reduction of silver.

XRD was commonly used for determined the chemical composition and crystal structure of a material; therefore, detecting the presence of silver nanoparticles in plant tissues can be achieved by using XRD by examining the diffraction peaks of the plant. The crystalline nature of Ag nanoparticles was also confirmed from X-ray diffraction (XRD) analysis. (V. Suseela *et al* .,2015) reported that biosynthesised silver nanostructure by employing *Indigofera hirsuta* L. plant leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image and the structural view under the scanning electron microscope. The XRD pattern showed three intense peaks in the whole spectrum of 2θ value ranging from 40 to 80. Average size of the particles synthesized was 15nm with size range 40 to 80nm with cubic and orthorhombic crystals.

The SEM images showing the high density silver nanoparticles synthesized by *Indigofera hirsuta* L.plant leaf further confirmed the development of silver nanostructures. The typical XRD pattern revealed that the sample contained a mixed phase (cubic and hexagonal) structures of silver nanoparticles. The average estimated particle size of this sample was 15 nm derived from the FWHM (full width at half maximum) of peak corresponding to 111 plane.

The present study of in reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Indigofera hirsuta* L.plant leaf extract as observed by the UV-Vis spectroscopy revealed the presence of silver nanoparticles . The XRD, SEM, TEM analyses showed the particle size between 25-50nm as well the cubic structure of the nanoparticles. FTIR analysis confirmed that the bio reduction of silver ions to silver nanoparticles was due to the reduction by capping material of plant extract. The present study, thus showed a simple green route for rapid and economical synthesis of silver nanoparticles

In the analysis by Energy Dispersive Spectroscopy of the AgNPs, the presence of elemental metal signals was confirmed. EDX analysis gives quantitative and qualitative status of elements that may be involved in formation of nanoparticles synthesized nanoparticle using *Indigofera hirsuta* L.plant aqueous leaf extracts and confirms the formation of silver nanoparticles. Spectrum also indicates some unidentified peaks in image which may be due to copper grid used for EDX analysis.EDAX results also show higher counts at 5 keV due to silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV dueto surface plasmon resonance.

The anti-bacterial activity was done on clinical pathogens *Escherichia coli*, *Vibrio cholera, Pseudomonas aeruginosa, Pseudomonas mirabillis, Salmonella typhi* by the standard agar diffusion method.

Habit Leaf extract and Silver nitrate synthesis.

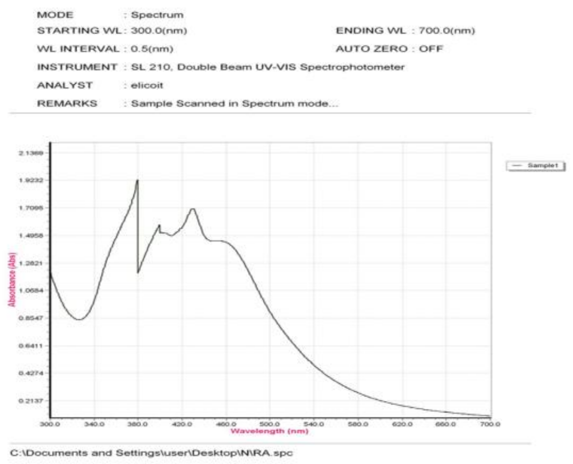

Fig 4: UV-Vis Spectrum.

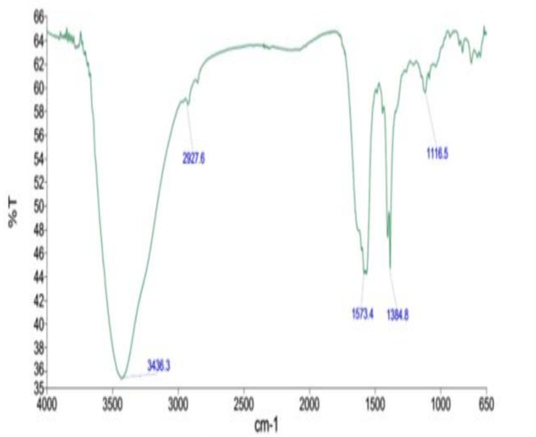

Fig. 5: Fourier Transform Infrared Spectrum.

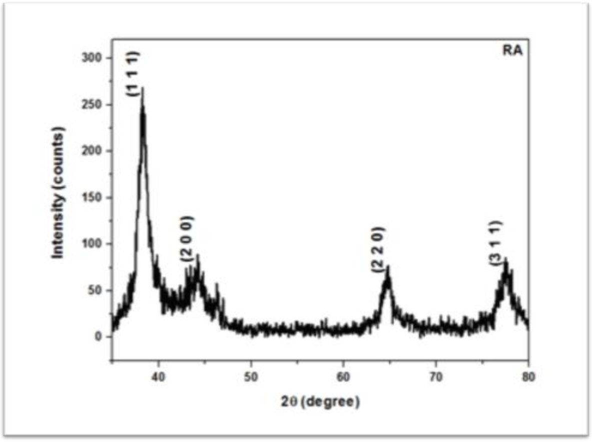

Fig. 6: X-Ray Diffraction patterns.

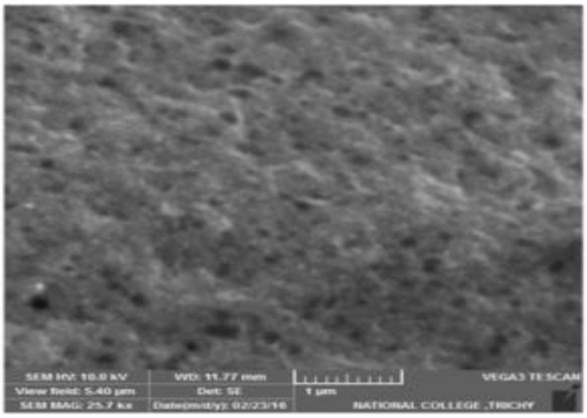

Fig. 7: Scanning Electron Microscopy Immage.

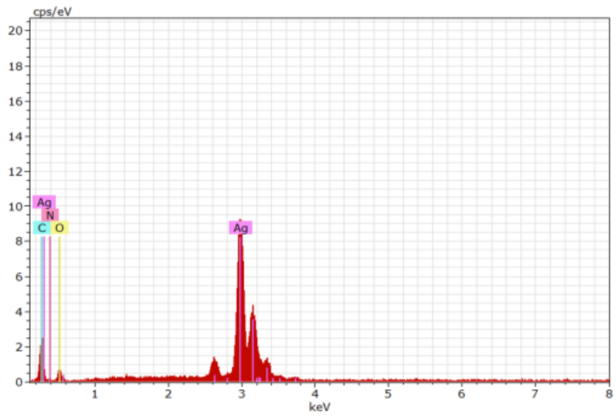

Fig. 8: Energy Dispersive X-ray Spectrum.

Table 4: Energy Dispersive X-ray Spectroscopy (EDAX).

E1 AN	Series	Unn. C (wt)	Norn. C (wt)	Aton. c (at)	(sigma) (wt)
Ag 47	L - Series	40.69	60.01	15.95	1.36
C 6	K - Series	12.99	19.16	45.74	2.67
O 8	K - Series	11.48	16.93	15.95	3.23
N 7	K - Series	2.64	3.90	7.97	1.37

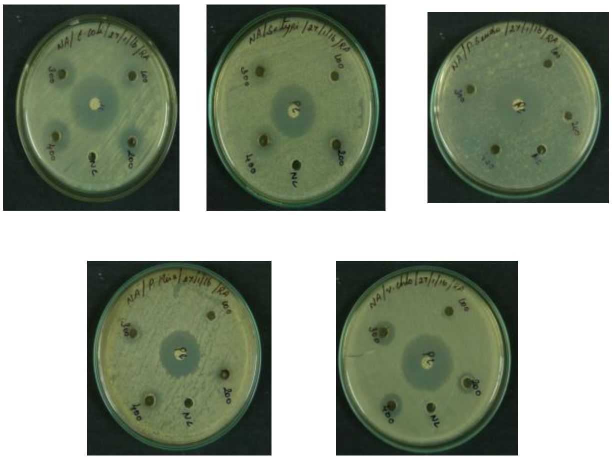

Fig. 9: Antibacterial activity of *Indigofera hirsuta L.* silver nanoparticles.

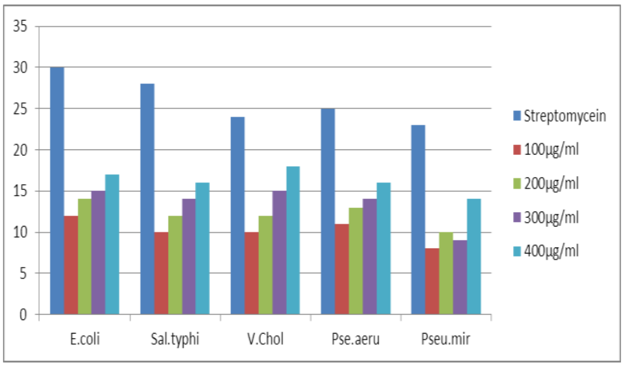

Fig. 5: Zone of Inhibition of *Indigofera hirsuta* L leaf extract synthesised Silver Nanoparticles.

FUTURE SCOPE OF RESEARCH

SUMMARY AND CONCLUSION

Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields.

The objective set for this study had been to prepare metal nanoparticles in a simple, cost effective and eco-friendly way unlike chemical procedures. We used the leaves extract of (*Indigofera hirsuta*) as a reducing and capping agent. By this method of preparation, the problems of environmental pollution were avoided. A long term research is required to overcome these limitations and implement this procedure for large scale productions. A few applications of these Green Silver Nanoparticles are: We proved the effective antibacterial property of these nanoparticles; hence we can think of its medicinal usage. Due to the highest conductive properties, we can implement these Silver nanoparticles in advanced portable gadgets. We can specifically use these nanoparticles in the production of clothing, leather items and coatings because it can protect these items from the attack of harmful microbes.

The silver nanoparticles synthesized by *Indigofera hirsuta*L. Plant leaf extract were characterized by UV-VIS spectrophotometer, FTIR, SEM, EDAX and XRD analysis. EDAX results also show higher counts at 5 keV due to silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV dueto surface plasmon resonance.Since there was presence of C,N and O, we have to go for LC-MS analysis for the organic molecules capped in it.

Our study confirms that the silver nanoparticles synthesized are effectively resistant to the growth and multiplication of clinical pathogenic bacteria like *Escherichia coli, Vibrio choleare, Pseudomoneas aeruginosa, and Proteus mirabilis.*

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