Comparative analysis of Silver Nanoparticles prepared from Different Plant extracts *(Hibiscus rosa sinensis, Moringa oleifera, Acorus calamus, Cucurbita maxima, Azadirachta indica)* through green synthesis method

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Life Science

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CERTIFICATE

This is to certify that the thesis entitled "*Comparative analysis of Silver Nanoparticles prepared from Different Plant extracts (Hibiscus rosa sinensis, Moringa oleifera, Acorus calamus, Cucurbita maxima, Azadirachta indica) through green synthesis method*" Submitted to National Institute of Technology, Rourkela for the partial fulfilment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by Ms. Sonali Pradhan under my supervision and guidance.

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DECLARATION

I hereby declare the thesis entitled **"Comparative analysis of Silver Nanoparticles prepared from Different Plant extracts *(Hibiscus rosa sinensis, Moringa oleifera, Acorus calamus, Cucurbita maxima, Azadirachta indica)* through green synthesis method"**, submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfilment of the Master Degree in Life Science is a faithful record of bonafide research work carried out by me under the guidance and supervision of Dr. Bismita Nayak, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

Date:
Place: Sonali Pradhan

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Place: Rourkela Sonali Pradhan

Date:

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Abstract

Green synthesis of nanoparticle is a novel way to synthesis nanoparticles by using biological sources. It is gaining attention due to its cost effective, ecofriendly and large scale production possibilities. In this present study five plants *Hibiscus rosa sinensis, Cucurbita maxima, Moringa oleifera, Azadirachta indica* and *Acorus calamus* were taken to investigate their potential for synthesizing silver nanoparticle. The silver nanoparticles synthesized were confirmed by their change of colour to dark brown due to the phenomenon of surface plasmon resonance. The characterization studied was done by UV-vis spectroscopy, Scanning electron microscopy (SEM), Atomic force microscopy (AFM), Dynamic light scattering (DLS) and zeta potential studies, X-Ray diffraction (XRD), Fourier Transmission infrared spectroscopy (FTIR). All the five plants synthesized silver nanoparticle show good antimicrobial activity against clinically important pathogens *Staphylococcus aureus, Klebsiella pneumoniae, Vibrio cholera and Escherichia coli.*

Keywords- Green synthesis, SEM, AFM, FTIR, DLS, XRD

1. INTRODUCTION

In recent days nanotechnology has induced great scientific advancement in the field of research and technology. Nanotechnology is the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering. Nanoparticle is a core particle which performs as a whole unit in terms of transport and property (Nour *et al.,* 2010). As the name indicates nano means a billionth or 10^{-9} unit. Its size range usually from 1-100nm (Nour *et al.,* 2010) due to small size it occupies a position in various fields of nano science and nanotechnology. Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. So the main aim to study its minute size is to trigger chemical activity with distinct crystallography that increases the surface area (Osaka *et al.,* 2006, Singh *et al.,* 2008 & Sinha *et al.,* 2009). Thus in recent years much research is going on metallic nanoparticle and its properties like catalyst, sensing to optics, antibacterial activity, data storage capacity (Nour *et al.,* 2010 & Sharma *et al.,* 2009).

The concept of nanotechnology emerged on 9^{th} century. For the first time in 1959, **Richard Feynman** gave a talk on the concept of nanotechnology and described about molecular machines built with atomic precision where he discussed about nanoparticles and entitled that *"There's plenty of space at the bottom"* (T.C *et al.,* 1974). **Professor Peter Paul Speiser** and his research group were first to investigate on polyacrylic beads for oral administration and target on microcapsule. In the year of 1960 nanoparticle develop for drug delivery and also vaccine purpose which change the medicinal scenario. The first paper published in1980 by **K. Eric Drexler** of Space Systems Laboratory Massachusetts Institute of Technology was titled as "*An approach to the development of general capabilities for molecular manipulation".* The term "nanotechnology" first time used as scientific field by **Nario Tanigushi** in the 1974 his paper was "Nanotechnology" mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule (Zhang and Webster, 2008).

Nanotechnology is a fast growing area in the field on science which is a interdisciplinary field of both science and technology that increase the scope of investing and regulating at cell level between synthetic material and biological system (Du.*et al.,* 2006 & Sinha *et al.*,2009). Nanotechnology proceeds by three processes - separation, consolidation, deformation of

material by one atom or molecule (Taniguchi 1974). It is divided into three types- **Wet nanotechnology** which deals with the biological system such as enzymes, membrane, cellular components. **Dry nanotechnology** deals with the surface science, physical chemistry & gives importance on fabrication of structure in carbon, silicon, inorganic materials. **Computational nanotechnology** which deals with modelling & stimulating the complex nanometre scale structure (Sinha *et al.,* 2009), these three fields are interdependent to each other. There are two methods of synthesis of metallic nanoparticles which are chemical method and physical method. In chemical approach it include chemical reduction(Guzman *et al.,* 2009), electrochemical technique(Rodriguez-Sanchez *et al.*2000), photochemical reduction (Balan *et al.,* & Sharma *et al.*, 2009).The chemical process is again subdivided into classical chemical method where some chemical reducing agent (such as hydrazine, sodium borohydride, hydrogen)are used, radiation chemical method generated by ionization radiation (Leff *et al.*, 1995; Lisiecki and Pileni, 1995; Huang *et al.,*1997;Gutierrez and Henglein, 1993; Nour *et al.*2010). In the physical approach it includes condensation (Raffi *et al.,* 2007), evaporation (Mitrakos *et al.,*2008) and laser ablation for metal nanoparticle synthesis (Zamiri *et al.,*2012). The biological synthesis of nanoparticle is a challenging concept which is very well known as green synthesis. The biological synthesis of nano material can solve the environmental challenges like solar energy conservation, agricultural production ,catalysis (Kumar *et al.,* 2011), electronic, optics (Evanoff *et al,* .2005), and biotechnological area (Soloviev and Mikhail 2007). Green synthesis of nanoparticle are cost effective, easily available, eco friendly, nontoxic, large scale production and act as reducing and capping agent(T.C *et al.,* 2011*)*in compared to the chemical method which is a very costly as well as it emits hazardous by-product which can have some deleterious effect on the environment (Kaler *et al.,* 2010). Biological synthesis utilizes naturally occupying reducing agent such as plant extract, microorganism, enzyme, polysaccharide which are simple and viable which is the alternative to the complex and toxic chemical processes (Du L. *et al.,* 2009). Plants can be described as nano factories which provide potential pathway to bioaccumulation into food chain and environment. Among the different biological agents plants provide safe and beneficial way to the synthesis of metallic nanoparticle as it is easily available so there is possibilities for large scale production apart from this the synthesis route is eco-friendly, the rate of production is faster in comparison to other biological models such as bacteria, algae and fungi (Nour *et al.,* 2010). From the various literature studies it can be stated that the amount of accumulation of nanoparticle varies with reduction potential of ions

and the reducing capacity of plant depends on the presence of various polyphenols and other heterocyclic compounds (Nair *et al.*, 2010)

Nanoparticle of gold, silver, copper, silicon, zinc, titanium, magnetite, palladium formation by plants has been reported. Colloid silver nanoparticle had exhibited distinct properties such as catalytic, antibacterial (Sharma *et al.*, 2009), good conductivity, and chemical stability. Silver nanoparticles have its application in the field of bio labelling, sensor, antimicrobial, catalysis, electronic and other medical application such as drug delivery (Jong *et al.*, 2008) and disease diagnosis.

2. REVIEW OF LITERATURE

Nanotechnology is a brainchild of modern fundamental science. It is a very complicated professional area, uniting the efforts of professionally qualified chemists, physicists, mathematicians, materials scientists, physicians, computer scientists, and so on. At the present stage nanoparticle research is an intense scientific research due to its wide potential application in biomedical, optical & electronic fields. Nanoparticles are a narrow bridge in between bulk materials and molecular (atomic) structures. Bulk materials have constant physical properties because they have grain structures with random grains individually oriented in space and contacting each other across grain boundaries but nanomaterials are made up of a single grain with all the atoms oriented in a crystalline lattice. (Sharma *et al.*, 2009) The main characteristics of nanomaterials that distinguish them from bulk materials are (1) large fraction of surface atoms; (2) high surface energy; (3) spatial confinement; (4) reduced numbers of imperfections that do not exist in the corresponding bulk materials (Cao 2004).Nanoparticles show different properties such as quantum confinement, Surface Plasma Resonance (SRP), decrease in melting temperature which are directly related to the crystal lattice of the nanomaterials. The use of Nanomaterials provide the following advantages, Firstly, as nanomaterials consist of very small particles they, promote accomplishment of super miniaturization and thus the nanostructures can be packed very closely together which can be useful for nanoelectronics. Secondly, because of their small dimensions, nanomaterials have large specific surface areas which increase the interactions between them and the environment in which they are located.

Nanoparticles can be broadly classified into two groups: Organic nanoparticles and Inorganic nanoparticles. Organic nanoparticle are carbon nanoparticle (fullerenes) and inorganic nanoparticles are magnetic nanoparticle, noble nanoparticle (gold and silver), semiconductor nanoparticle (titanium oxide and zinc oxide). Especially inorganic nanoparticles have created attention towards itself due to its superior material properties with versatile functions. Due to nano size feature it easily used for chemical imaging drugs agents and drug. Its versatile function used for the cellular delivery as they are widely available, rich functionality, good biocompatibility. This is also a good carrier of targeted drug delivery and controlled drug release (Xu *et al.*, 2006). it is a completely advantageous material foe medical science For example mesoporous silica combined with molecular medicines shows a excellent image on

drug releasing. Gold nanoparticle is good carrier in thermo therapy of biological target (Cheon & Horace, 2009). Silver nanoparticle shows antimicrobial activity which heals the wounds and infectious disease (Ravishankar and Jamuna, 2011).Synthesis of nanoparticle gets concern in nanotechnology due to the variable size, shapes, chemical composition & controlled dispersity and their potential use in the medical science for the better treatment of human benefits.

Traditionally, researchers generally used two methods for the synthesis nanoparticles such as **Bottom-up approach**: The bottom-up approach is a nano-architectural phenomenon of self assembly of materials from cluster-to-cluster, molecule-to-molecule or atom-to-atom on top of a base substrate. The main concern in the bottom-up approach is the adhesion of the surface layers to the base substrate. The most commonly used bottom-up methods are wielding & riveting.

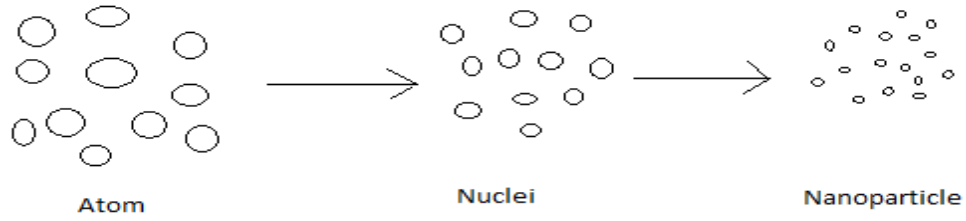

Fig 1: Bottom High approach.

Top down approach: the Top down method refers to a set of fabrication technologies starting with a block bulk material which share the same material with the base substrate. The most commonly used top down methods are milling, drilling and grinding.

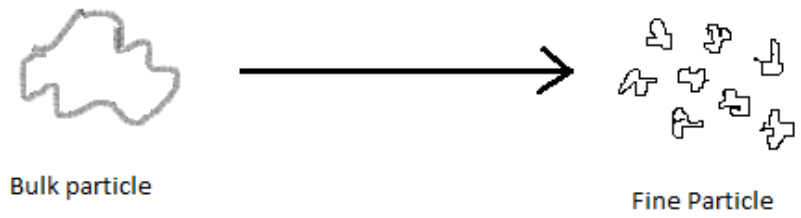

Fig 2: Top-down approach.

2.1: Physical and Chemical approach for synthesis of nanoparticles

2.1.1: Physical approach:

In physical approach metal nanoparticles are synthesised by either evaporation - condensation method or laser ablation method. In evaporation condensation method the reaction is carried out using a tube furnace at atmospheric pressure. The target material is kept within a boat centred at the furnace is vaporized into a carrier gas. Gutav *et a.*, Kruis *et al.*, Magnusson et al, has successfully synthesized Ag, Au, Pb and fullerene nanoparticle by using evaporation method. But this method has some drawbacks such as the tube furnace occupies a large space, consumes a great deal of energy raising the surrounding temperature around the source material and requires a lot of time to achieve the thermal stability. (Kholoud *et al.,* 2010) Mafune *et al.,* 2000 ; Kabashin and Meunier, 2003; Sylvestre *et al.,* 2004; Tsuji *et al.,* 2002, Compagnini *et al.,* 2003; Chen and Yeh, 2002; Dolgaev *et al.,* 2002 synthesised silver nanoparticle synthesis by laser ablation method. The particles synthesised through laser ablation method depends upon the wavelength of the laser, the duration of the laser pulses the laser fluence, the ablation time duration and the effective liquid medium which may or may not containing the surfactant.

2.1.2: Chemical approach:

Chemical method is the most commonly used method for the synthesis of silver nanoparticles. The most commonly used reducing agents are sodium borohydride, hydrazine hydrate, potassium auro chlorate and sodium citrate. The reduction of various complexes with Ag+ ions leads to the formation of silver atoms (Ag0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles.

The function of the protective agent is to protect the nanoparticles from agglomeration (Oliveira *et al.,* 2005; Bai *et al.,* 2007). The most commonly used protecting agents are poly (vinyl pyrolidone) (PVP), poly (ethylene glycol) (PEG), poly (methacrylic acid) (PMAA) and poly(methylmethacrylate) (PMMA)

Sol-gel technique is used for the synthesis of metal oxides from a chemical solution which acts as a precursor for integrated network of discrete particles or polymers. The precursor sol

can be either deposited on the substrate to form a film cast into an appropriate container having desired shape or can be used to synthesize powders.

Solvothermal synthesis is a flexible low temperature route in which polar solvents under pressure and at temperatures above their boiling points are used. The reaction of the reagents under the solvothermal conditions increases significantly and enabling the reaction to take place at lower temperature.

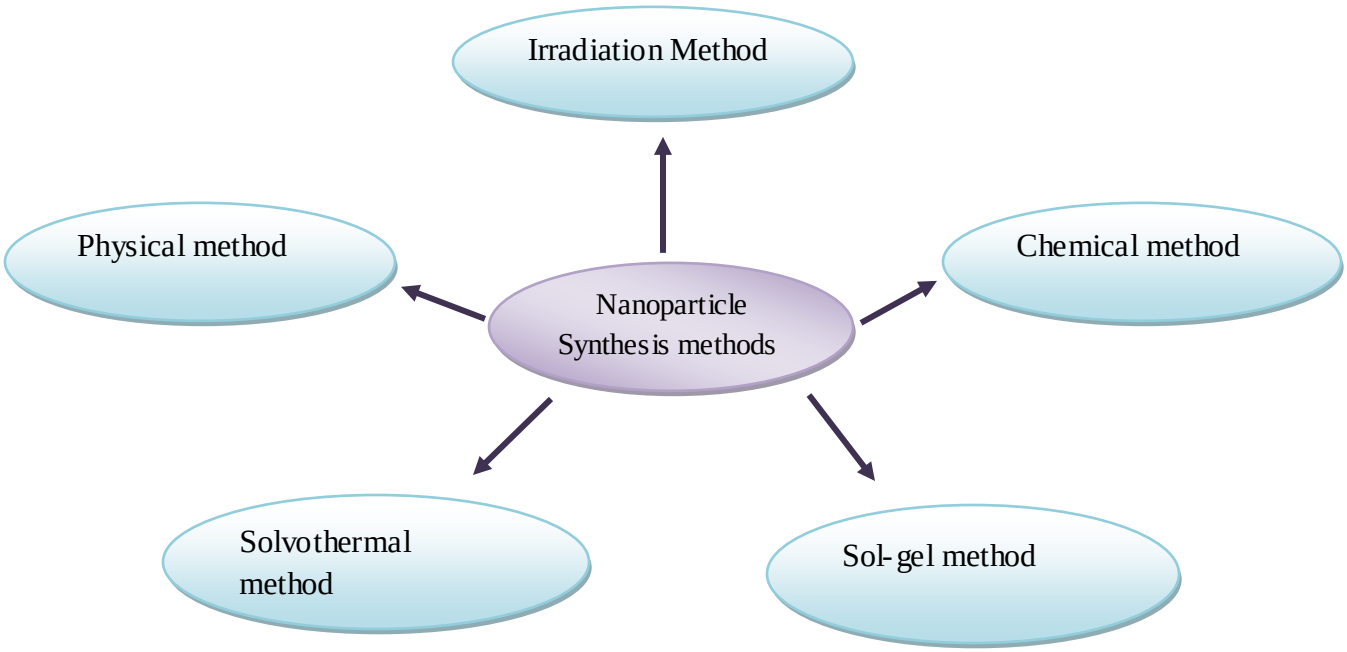

Fig 3: Different methods of nanoparticle synthesis.

2.2: Biosynthesis of nanoparticles:

Although chemical & physical methods are very successful to produce well- defined nanoparticles, they have certain limitations such as increase cost of production, release of hazardous by-products, long time for synthesis and difficulty in purification (Nagajyothi and Lee, 2011). Global warming & climate change has induced a worldwide awareness to reduce the toxic & hazardous waste materials, thus, the green synthesis route have raised actively the progress in the fields of science & industry (Ahmad *et al.,* 2011). Biosynthesis of nanoparticles as the name indicates help in the synthesis of very complex reaction within a fraction of minutes have now taken up the attention towards synthesis grievance the need of environmentally benign technologies in material science (Harekrishna *et al.,* 2009). Use of

biological organisms such as microorganism, plant extracts and biomass could be a best alternative method of physical and chemical method for synthesis of nanoparticles because the biological or green synthesis route is very spontaneous, economic, environmental friendly and non-toxic. Therefore, biological sources such as bacteria, fungi, yeasts, algae and plants can materials catalyzed specific reaction as a part of modern & realistic biosynthetic strategy. The current prospective on biological system has created a commercial importance due to their enzymatic reactions, photochemical characteristics and herbal nature. Biological system has created a specific and revolutionary change for the synthesis of nanoparticles due to their mode of mechanism trough which the bio reduction of the metallic salts occurs is still a mystery. Numerous researches have been done on the synthesis of nanoparticles from biological system of for their application in the field of biomedical, pharmaceutical, cosmetic and environmental use. Bio fabricated nanoparticles can be used for bioremediation purpose because nanoparticles can diffuse or penetrate through the contaminants and cause a redox reaction to clean the surface materials. Nature has some processed device to synthesis of nano and micro sized materials which contribute to the development of relatively new and unexplored area of research based on the biosynthesis of nanomaterials.

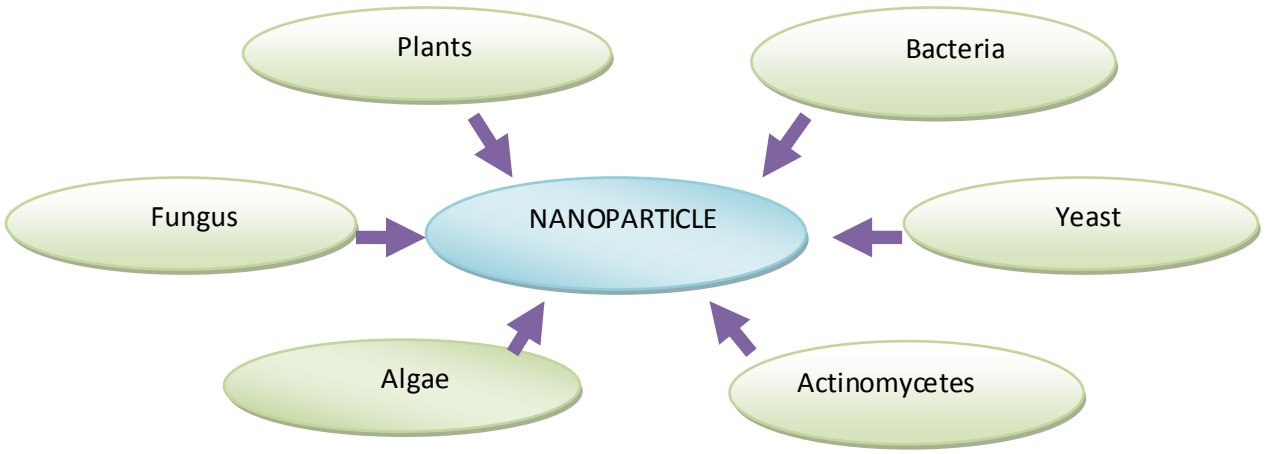

Fig 4: Different routes for biosynthesis of nanoparticles.

2.2.1: Bacteria:

Many microorganisms can synthesise inorganic nanoparticles like silver, gold, magnesium, cadmium sulphide and silicon oxide nanoparticles. The resistance caused by the bacterial cell for silver ions in the environment is responsible for its nanoparticles synthesis (Saklani *et al.*, 2012). It has been reported earlier that *Bacillus subtilis* 168 has the ability to reduce Au^{3+}

ions to produce octahedral gold particles of 5–25 nm within bacterial cells by incubating the cells with gold chloride (Beveridge and Murray 1980) under ambient temperature and pressure conditions. *Pseudomonas stutzeri* AG259 the silver resistance bacterial strain can accumulate silver nanoparticles, along with some silver sulfide, in the cell where particle size ranges from 35 to 46 nm (Slawson *et al.,* 1992). *Lactobacillus*, a common bacterial strain present in the buttermilk, synthesizes both Au and Ag NPs of well-defined morphology under standard conditions. Nair *et al.,* 2002 and Shahverdi *et al.,* 2007 reported the synthesis of metallic nanoparticles of Ag using the cultural supernatants of *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae*. Most of the metal ions have toxic effect on bacteria so the reduction of ions or the formation of water insoluble complexes is a defence mechanism developed by the bacteria to overcome such toxicity (Sastry *et al.*, 2003). It is generally believed that the enzymes of the organisms play a key role in the bioreduction process but some studies have shown contradictory results. Fu *et al* have reported dried cells of *Bacillus megaterium* D01, *Lactobacillus sp*. A09 could reduce silver ions where the processes of bioreduction were probably non enzymatic. There silver ions were reduce by the interaction of the silver ions with the groups on the cell wall of the microorganisms The most widely acknowledged mechanism for the biosynthesis of silver nanoparticles is the presence of the enzyme nitrate reductase which converts nitrate into nitrite. During *in vitro* synthesis of silver using bacteria, the presence of alpha-nicotinamide adenine dinucleotide phosphate reduced form (NADPH)- dependent nitrate reductase would remove the downstream processing step thus during the reduction, nitrate is converted into nitrite and the electron is transferred to the silver ion causing the silver ion to reduced silver. This has been observed in *Bacillus licheniformis* which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts Ag^{+} to Ag^{0}.

2.2.2: Fungi:

Fungi can produce larger amounts of nanoparticles in comparison to bacteria because they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles (Mohanpuria *et al.,* 2008). Fungi can be described as the best nano-factories in relation to bacteria because they have high binding capacity with metal ions in intracellular region, they are easy to culture on solid substrate fermentation, they can grow on the surface of inorganic substrate during culture leading to efficient distribution of metals as catalyst. The advantage of the production of nanoparticles extracellularly from fungi is that a large

quantity of enzyme which are in pure state and free from cellular protein can be easy to apply for the simple downstream process. Ahmad *et al.*, 2003 reported the synthesis of fabrication of extremely stable Ag hydrosol by using *Fusarium oxysporum* where the particles were stabilized by the proteins excreted through the fungus. Bhainsa *et al* has reported the extracellular biosynthesis of Ag particles in the 5-25 nm range using *Aspergillus fumigates*. Vigneshwaran *et al.*, 2006 reported the biomimetics of Ag nanoparticles by using *Phaenerochaete chrysosporium* commonly known as White rot fungus. Basavaraja *et al*, fabricated spherical and stable Ag nanoparticles in the range of 10-60 nm by using *Fusarium semitectum*. Varshney *et al.*, 2009 reported synthesis of Ag nanoparticles in the range of 20-80 nm by using a novel fungi *Hormoconis resinae*.

The possible mechanism for the synthesis of silver nanoparticle by fungi is said to follow the following steps: trapping of Ag^+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system (Mukherjee *et al* ., 2001)

2.2.3: Actinomycetes:

Actinomycetes are microorganisms that share some of the important characteristics of fungi and bacteria. Due to their ability to produce secondary metabolites such as antibiotics actinomycetes are now getting focus for the synthesis of metallic nanoparticles. Sastry *et al.*, 2003 reported the synthesis of Au nanoparticles by using the extremophilic actinomycete, *Thermomonospora* sp which yielded polydisperse Au nanoparticles. Ahmad et al reported the intracellular synthesis of Au nanoparticles by using alkalotolerant *Rhodococcus* sp. They observed that the concentration of nanoparticles were more on the cytoplasmic membrane than on the cell wall. This could be due to reduction of the metal ions by enzymes present in the cell wall and on the cytoplasmic membrane but not in the cytosol.

2.2.4: Algae:

Algae are a diverse group in the plant kingdom that are being explored for their application in nanotechnology. Hosea *et al* reported the synthesis of Au nanoparticles on the alga *Chlorella vulgaris*. Lengke et al reported the synthesis of Au nanoparticles having controlled shape by using the blue-green algae *Plectonema boryanum* by treating them with aqueous Au $(S_2O_3)_{23}$ and $AuCl_4$ solutions. Singaravelu *et al.*, 2007 reported the rapid formation of Au nanoparticles through extracellular biosynthesis in marine alga *Sargassum wightii*. Scarano

and Morelli reported the fabrication of phytochelatin coated CdS nano crystals by using the phytoplanktonic alga *Phaeodactulum tricornatum*. Konishi *et al.*, 2007 reported the synthesis of Pt nanoparticles of 5 nm from aqueous $PtCl_6^{2-}$ at neutral pH under room temperature by using *Shewanella* algae.

2.2.5: Plants:

Various microorganisms such as bacteria, algae, fungi and yeasts are used for the biosynthesis of nanoparticles but recently a new trend has come to force i.e., the use of plants for the fabrication of nanoparticles because of its spontaneous, economical, eco-friendly protocol, suitable for large scale production and single step technique for the biosynthesis process (Huang *et al.,* 2007). The main mechanism considered for the synthesis of nanoparticles mediated by the plants is due to the presence of phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides (Prabhu *et al.,* 2012). A number of plants are being currently investigated for their role in the synthesis of nanoparticles such as *Cinnamomum camphora* leaf (Huang *et al.,* 2007), *Pelargonium graueolens* leaf (Shankar *et al.,* 2003), *Azadirachta indica* leaf (Shankar *et al.,* 2004), *Emblica officinalis* leaf (Ankamwar *et al.,* 2005), *Aloe vera* leaf (Chandran *et al.,* 2006), *Alfalfa* sprouts (Gardea-Torresdey *et al.,* 2003), *Helianthus annus, Basella alba, and Saccharum officinarum* (Leela *et al.,* 2008), *Carica papaya* callus (Mude *et al.,* 2009), *Jatropha curcas l*eaf (Bar *et al.,* 2009), *Eclipta* leaf (Jha *et al.,* 2009), *Glycine max* (soybean) leaf (Vivekanandan *et al.,* 2009), *Coriandrum sativum* leaf (Sathyavathi et al 2010), *Syzygium cumini* leaf (Kumar et al 2010), *Cycas* leaf (Jha *et al.,* 2010), *Argimone mexicana* leaf (Khandelwal *et al.,* 2010), *Allium cepa* (Saxena *et al.,* 2010), *Stevia rebaudiana* leaves (Varshney *et a.,l* 2010), *Solanum torvum* (Govindaraju *et al.,* 2010), *Zingiber officinale* (Singh *et al.,* 2011), *Capsicum annuum* (Li *et al.,* 2007), *Dillenia indica* fruit (Singh *et al.,* 2013), *Alternanthera sessilis* (Niraimathi *et al.,* 2013), *Morinda citrifolia* (Suman *et al.,* 2013), *Phytolacca decandra, Gelsemium sempervirens, Hydrastis canadensis and Thuja occidentalis* (Das *et al.,* 2013) (Pinus desiflora), *Diopyros kaki, Ginko biloba, Magnolia kobus and Platanus orientalis* (Song *et al.,* 2009), *Ulva fasciata* (Rajesh *et al.,*2012)

Flow chart of the synthesis of nanoparticles

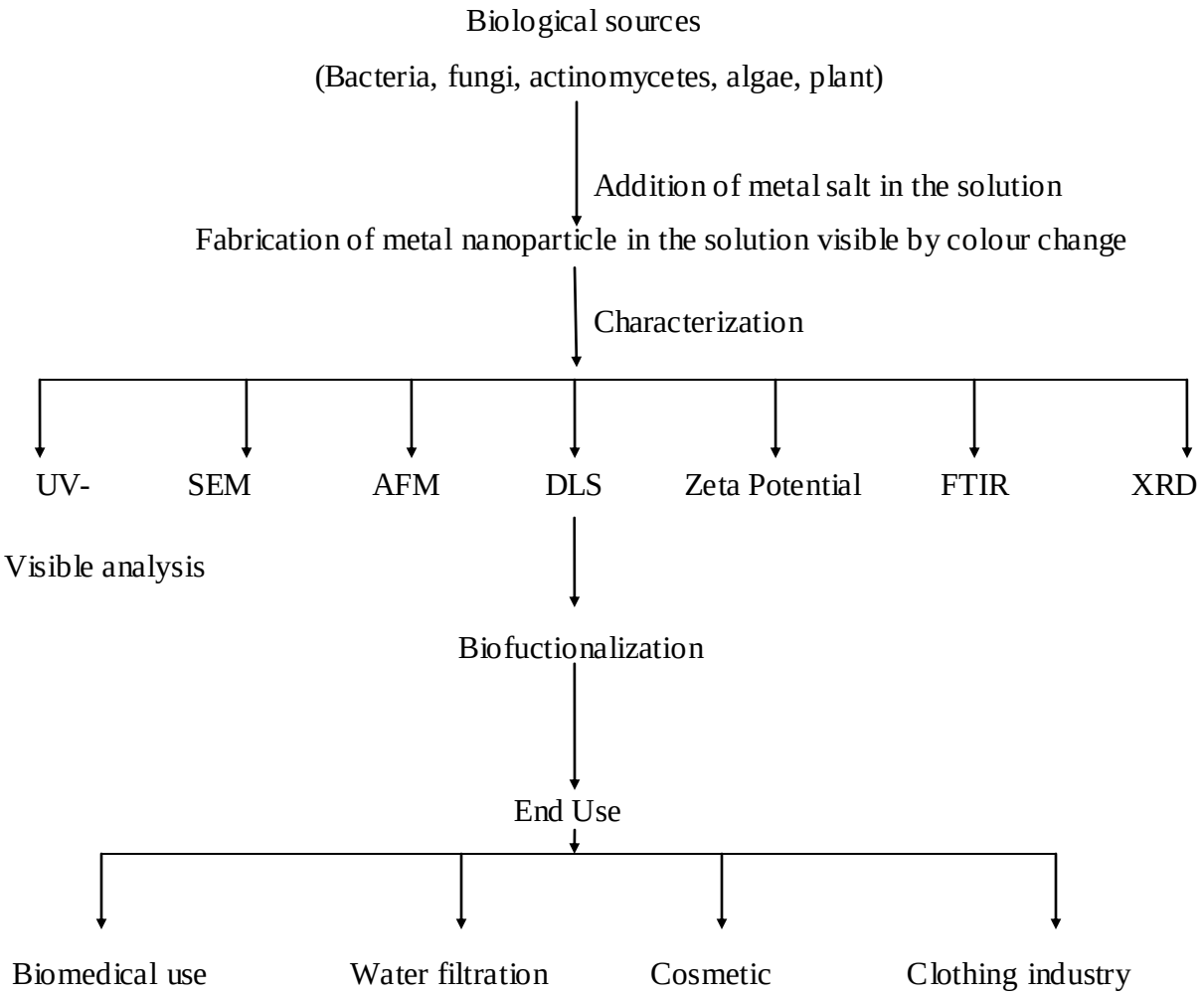

Fig 5: General procedure for synthesis of nanoparticles.

Table 1: Synthesis of different nanoparticles from biological sources.

Biological Sources	Nanoparticle Produced	Nanoparticle Size	References
Plants:			
• *Medicago sativa*	Au nanoparticle	4-10nm	Gradae *et al.*, 2002
• *Chilopsis linearis*	Au nanoparticle	1.1nm	Armendariz *et al.*, 2004
• *Pelargonium graveolens*	Au nanoparticle	21-70nm	Shankar *et al.*, 2003
• *Triticum aestivum*	Au nanoparticle	10-30nm	Armendariz *et al.*, 2004
• *Sesbania*	Au nanoparticle	6-20nm	Sharma *et al.*, 2007
• *Medicago sativa*	Ag nanoparticle	2-20nm	Gardea *et al.*, 2003
• *Azadirachta indica*	Ag nanoparticle	50-100nm	Shankar *et al.*, 2004

Bacteria: • *Bacillus subtilis* • *Pseudomonas aeruginosa* • *Rhodopseudomonas capsulate* • *Escherichia coli* • *Desulfovibrio desulfuricans* • *Pseudomonas stutzeri* AG259 • *Klebsiella pneumonia* • *Bacillus licheniformis* • *Clostridium thermoaceticum* • *Rhodopseudomonas palustris* • *Desulfobacteriaceae*	Ag nanoparticle Au nanoparticle Au nanoparticle Au nanoparticle Pd nanoparticle Ag nanoparticle Ag nanoparticle Ag nanoparticle CdS nanoparticle CdS nanoparticle ZnS nanoparticle	5-60nm 15-30nm 10-20nm 20-25nm 20-50nm 200nm 1-6nm 40nm 20-200nm 0-0.25nm 2-5nm	Saifuddin *et al.*, 2009 Husseiny *et al.*, 2007 He *et al.*, 2008 Deplanche & Macaskie,2008 Deplanche *et al.*, 2008 Joerger *et al.*, 2000 Mokhtari *et al.*, 2009 Kalishwaralal *et al.*, 2008 Cunningham & Lundie,1993 Bai *et al.*, 2009 Labrenz *et al.*, 2000
Yeast: • Yeast MKY3 • *Candida glabrata* • *Schizo saccharomyces Pombe* • *P. jadini*	Ag CdS CdS Au	2-5nm 20Å 1-1.5nm Few to 100nm	Kowshik *et al.*, 2003 Haverkamp *et al.*, 2007 Kowshik *et al.*, 2002 Konishi *et al.*, 2006
Algae: • Diatoms6 • Sargassum alga	SiO2	50-100nm	Singaravelu *et al.*, 2007
Fungi: • *Aspergillus fumigates*	Ag	5-25nm	Armendariz *et al.*, 2004

• *Fusarium semitectum* • *Verticillum sp.*	Ag Ag	20-25nm 20-25nm	Bhainsa *et al.,* 2006 Mukherjee *et al.,* 2001
Actinomycetes: • *Thermospora sp* • *Rhodococcus sp*	 Au Au	 8nm 5-15nm	 Ahmad *et al.,* 2003 Gericke *et al.,* 2006

2.3: Silver Nanoparticles:

Silver nanoparticles have attracted and demandable research of interest in the field of nanotechnology, due to its distinct properties such as good conductivity, chemically stable, catalytic activity, surface enhanced Raman scattering and antimicrobial activity (Li. Z *et al.,* 2006; Chen Y.Y *et al.,*2005; Setua P *et al.,*2007). Silver is widely used as catalyst for the oxidation of methanol to formaldehyde and ethylene oxide. Due to colloidal nature it use as substrate for surface enhanced spectroscopy, as it partly require electrical conducting surface. In this era silver is use as antimicrobial agent. Recent focuses towards silver nanoparticle synthesis for increasing the treat of antibiotic resistance, caused by the misuse of antibiotic. (Panaek *et al.,* 2006; Sandbhy *et al.,* 2006). Several hypotheses have been found to the antimicrobial activity of silver nanoparticle. Silver nanoparticle have capacity inactivates bacterial enzyme by releasing ionic silver which inactivates the thiol groups. This silver ions inhibits bacterial DNA replication, damage cell cytoplasm, depleting levels of adenosine triphosphate (ATP) and finally death of cell (Feng *et al.,* 2000). As the nanoparticle distinct property surface to volume ratio silver nanoparticle increases surface to contact with bacterial cell which promote silver ion to dissolve and improving the bacterialsidal effectiveness (Stobie *et al.,* 2008).Chemical reduction is most commonly used for synthesis of silver nanoparticle. For the chemical reduction process some reductants are used such as borohydride, citrate, ascorbate and elemental hydrogen. (Maribel *et al.,* 2009). By the reduction of silver ion in aqueous solution, a nanosized colloidal silver particle formed. During chemical reaction various complexes with Ag^{+} ions leads to formation silver particle (Ag^{0}), followed by the agglomeration into oligomeric clusters, which leads to formation of colloidal silver nanoparticle. The first observation of nanoparticle synthesis is colour change of the aqueous solution, if wavelength of the solution is from 380-400nm then smaller nanoparticle is formed. This band formation after nanoparticle synthesis is due to collective

oscillation or excitation of electron in the solution (i.e. surface plasmon resonance) (Yamini *et al.*, 2011). Borohydride is a stronger reductant which synthesized smaller monodispersed particle but difficult to controlled larger size. Use of citrate is a weaker reductant rate of synthesis is slower and particle size is not so small. In the chemical reduction process a stabiliser is most use that prevent agglomeration colloidal particle. Plants like biological sources (bacteria, fungus, algae, yeast) are used for synthesis of silver nanoparticle by using chemical sodium nitrate (R.Geethalakshmi *et al.*, 2010). The green synthesis of silver nanoparticle are mainly three steps are considered: selection of solvent medium, selection of biological source related reducing agent, selection of nontoxic stabilizing agents. *Acalypha indica* leaves synthesized 20-30nm silver nanoparticle in chemical reduction method (Krishnaraj *et al.*, 2010).

2.4: Plant Samples:

2.4.1: *Moringa oliefera*:

Moringa oliefera is a commonly found plant, which is dispersed in many countries of the tropics and subtropics. It's all over parts (roots, seed, bark, leaves, fruit, and immature pods, flowers) are highly nutritious with medicinal value. The whole plant contains a sketch of important minerals, and proteins, vitamins various phenolic compound, amino acid and very important β – carotene which is found in high amount (Farooq *et. al.*, 2007). This plant carries many phytochemical such as zeatin, kaempferom, quercetin with various combinations. This plant has highly medicinal effect on curable diseases such as cardiac and circulatory drugs, possess antitumor (Makonnen *et. al.*, 1997), antipyretic, antioxidant, antidiabetic, antiulcer, anti inflammatory, antiepileptic (Pal *et. al.*, 1995). Other possible medicinal propreties are antispasmodic (Caceres *et. al.*, 1992), diuretic (Morton, 1991), antihypertensive (Dahot, 1988), lowering of cholesterol level (Mehta *et. al.*, 2003), antioxidant, antidiabetic, (Ruckmani *et. al.*, 1998), antibacterial and antifungal activities (Nickon *et. al.*, 2003). *M.oleifera* seed act as water purifier against Gram positive and Gram negative bacterial cells in the powder form (Muyibi and Evison, 1995; Olsen, 1987; Broin *et. al.*, 2002; Kawo, 2007). Its seeds have also capacity to biodegrade to heavy metals (Sharma *et. al.*, 2006). Other components of M.olaeifera employed as anticancerous and hypo-tensive are 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy)benzyl isothiocy-anate , 4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate.(Abuye C *et. al.*, 1999), niazimicin (Akhtar AH *et. al.*, 1995), pterygospermin (Bell PC *et. al.*, 1986), benzyl isothiocyanate (Anwar F *et. al.*, 2003), and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (Asres K, 1995).

2.4.2: *Cucurbita maxima*:

Cucurbita maxima are well known as pumpkin. It is an herbaceous plant having medicinal value.It contains naturally active components with polysaccharides, fixed oils, para-aminobenzoic acid, peptides, sterol, and proteins (Buchbauer G *et. al.*, 1998). The phytochemical found in fruits and leaves are polysaccharides, phenolic glycosides, and 11E-octadecatrienoic acid speciall fruits contains carotenoid and γ -aminobutyric acid (Koike K *et. al.*, 2005; Murkovic M *et. al.*, 2002). Hypoglycemic a novel chemical found in Cucurbita maxima containing 8.48% sugar(polysaccharides) which reduce blood glucose and an upsurge in plasma insulin and protected the diabetic nephropathy(Zhang YJ *et. al.*, 2002; Ju LY *et. al.*, 2001).Blood glucose level, Hypoglycemic activity, serum total cholesterol level and triglyceride induced in diabetic rabbits when applied with pumpkin powder (Zhang ZJ 1998)

2.4.3: *Azachata indica*:

Neem is a natural medicine since ancient time in Ayurved. All parts of neem plant (leaf, seed, fruits, bark, and flower) are useful for medicinal value. It has many propreties as anti bacterial, anti fungal, anti helmintic, anti diabetic contraceptic and sedative. Neem has effect on degenerative diseases such as Diabetes, Arthritis, Rheumatism, Cancer and Chronic Fatigue. It also shows effect on Tuberculosis, Bronchitis, Conjunctivitis, Allergies, Stress, and Insomnia. The phytochemicals present in neem are alkaloids, quinines, resins, tannins, flavanoids, fats, saponins, phenolic compounds, Proteins and carboxylic acids (Khan *et al.*, 2010).

2.4.4: *Hibiscus rosa sinensis*:

Hibiscus is an ornamental plant traditional used as for anti-inflammatory, demulcent, aphrodisiac, refrigerant, anodyne, laxative, emollient (J. Anjaria, *et. al.*, 2002). The phytochemicals found in this are medicinally steroids, flavonoids, tannins, reducing sugar, anthocyanin pigment, carotene, thiamine, riboflavin, niacin and ascorbic acid (kumar *et al.*, 2012). It has various activities like antitumor, antidiarrheal, antiestrogenic antispermatogenic, androgenic, antiphologistic (Kholkute SD and Udupa KN 1976), antiimplantation (Murthy DRK *et. al.*, 1997), wound Healing anticonvulsant (Nayak *et. al.*, 2007).

2.4.5: *Acorus calamus*:

A. calamus has an existence in history as a traditional medicine in India and generally is known as sweet flag, sweet grass and sweet cane (Family: Acoraceae). Root and rhizome of *A. calamus* used for medicine. It has beneficial role in improved learning performance or increase memory power and anti-aging effect (Nishiyama *et. al.*, 1994). This act as a anti-stressor and also antioxidative capacity which in turn could be achieved by protection of decreasing GSH and restoring free radical scavenger‘s enzymatic activity. (Sundaramahalingam *et. al.*, 2005). In Ayurveda it used on counter the side effects of all hallucinogens. The rhizome alcoholic extract has sedative and analgesic properties and causes depression in blood pressure and respiration rate and also used to treat intestinal coli, gastritis and gastric ulcers (Desai *et. al.*, 1984).

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2.5: Characterization of Silver Nanoparticles:

The characterization study of silver nanoparticle was done by the examining size, shape and quantity of particles. Number of technique is used for this purpose, including UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Fourior Transmission Infrared Spectroscopy (FTIR), X-Ray Diffaction (XRD), and Dynamic Light Scattering (DLS).

2.5.1: UV-vis Spectroscopy:

Absorbance spectroscopy is used to determine the optical properties of a solution. A Light is send through the sample solution and the amount of absorbed light is measured. When the wavelength is varied and the absorbance is measured at each wavelength. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law. The examination of nanoparticles, the optical properties are much more complicated. For instance, the measured absorbance spectrum does not necessarily show the actual absorbance but the extinction of the light is both the absorbed and the scattered light from the particles. These wave lengths arise due to the surface Plasmon resonance of the particle.

2.5.2: Scanning Electron Microscope:

Scanning electron microscope (SEM) analysis the employed to characterization of size, shape & morphologies of formed nanoparticle SEM gives high-resolution images of the surface of a sample is desired. The scanning electron microscope works as same principle as an optical microscope, but it measures the electrons scattered from the sample rather than photon.

Because electrons can be accelerated by an electric potential, the wavelength can be made shorter than the one of photons. This makes the SEM capable of magnifying images up to 200.000 times. At the same time it is possible to achieve high resolution pictures of the surface, making the instrument very useful in determining the size distribution of nanoparticles.

2.5.3: Atomic Force Microscope:

The AFM is an instrument capable of measuring the topography of a given sample. A nanosized tip attached on a cantilever is traded over the sample and a 3D image of the sample topography is generated on a computer. The advantage of the AFM over SEM is the ability to make topographical measurements for detection and investigation of the size and shape of silver nanoparticles in three dimensions. The AFM generally measure the height of silver nanoparticle.

2.5.4: Dynamic Light Scattering:

The DLS technique uses light to determine the size of particles in a solution. Light at a given frequency is sent through the solution from a laser. When the light interacts with the moving particles in the solution and is scattered, the frequency of the light is also changed. This change of light frequency is directly related to the size of the particles in the solution; the smaller the particles, the greater the shift in the light frequency. This difference in the light shift is used to determine the size of the particles in the solution. DLS is capable of measuring particles in the size range from a few nanometers to a few micrometers. It is therefore applicable for determining the size of silver nanoparticles.

2.5.5: Fourier Transmission infrared spectroscopy:

FTIR is a chemical analytical method which measures infrared intensity v/s wavelength or wave number of light. It used to analysis of possible bio molecule and also bonding interaction between themselves. IR spectroscopy detects the vibration characteristics of chemical functional groups of the sample. When an infrared light interacts with matter, chemical bonds will shows stretch, contract and bend form. These chemical functional group tends to adsorb infrared radiation in a specific wave number range of the structure of the rest of the molecule. The silver nanoparticle synthesis, FTIR data measures interaction between

Ag salts and proteins molecules, which accurate for the reduction of silver ions and stabilization of Ag NPS formed.

2.5.6: X-Ray Diffraction:

XRD is a technique to used go study phase composition of a sample, crystal structure, texture or orientation. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffracted light at different angle. When X-ray passing through a crystal it produces a diffraction pattern, that diffraction gives the information about the atomic arrangement within the crystals. In silver nanoparticle XRD gives phase structure and purity of the particle.

3. Biological Samples

3.1: *Hibiscus rosa sinensis*

Classification:

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dilleniidae
Order : Malvales
Family : Malvaceae
Genus : *Hibiscus*.
Species : *rosa sinensis*

Fig 6: *Hibiscus rosa sinensis*

Characteristics:

H. sinensis is a traditional medicinal plant to Indian and Chinese herblogy. It is a flowery plantsm, the flowers are large, petals are thin, trumpet-shaped, with five or more petals different type of colour found in this plant species that is white to pink, red, orange, purple or yellow. Its roots are cylindrical 5-1 cm in length and 2 cm in diameter. The leaves are simple ovate and entire base and coarsely toothed at the apex. It carries five petals with long stamen. It contains vitamin E and various minerals. It is used as antifungal agent prevents premature greying, hair loss and scalp disorders.

3.2: Cucurbita maxima

Classification:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Dilleniidae
Order	: Violales
Family	: Cucurbitaceae
Genus	: *Cucurbita*
Species	: *maxima*

Fig7: *Cucurbita maxima*

Characteristics:

They are large low creeping vine having very large leaves those are palmate with a maple shape having small sharp serrations along the margin; flowers are very large bright yellow with messy edge; fruit is a large almost round in shape. Pumpkin seeds are also useful, it contain sterols, vitamin E, fatty acids and non-protein amino acids. It use as pharmaceutically treating disease like rheumatism, bladder disorders, wounds, benign prostatic hyperplasia, and certain female reproductive complaints. Pumpkin seeds also possess vitamin B, and many essential minerals such as iron, zinc, and they are very nutritious and stimulating. Other nutrients found in *Cucurbita* seeds are magnesium, phosphorus, niacin, folic acid, riboflavin, thiamine and antioxidants. Zinc helps the healing process generally useful in treating the enlarged prostate gland and pantothenic acid helps to be in good health.

3.3:Moringa-oliefera

Classification:

Kingdom :Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dilleniidae
Order : Capparales
Family : Moringaceae
Genus : *Moringa*
Species : *oleifera*

Fig 8: *Moringa oleifera*

Characteristics:

Moringa oleifera is a tall, thin, fast growing evergreen tree. The tree grows to maximum 8 m high and diameter about 60 cm dbh. The bark is thick, soft, corky and dark grey colour, leaves are alternate (the old ones soon falling off; each leaf large), with opposite pinnae, spaced about 5 cm apart up the central stalk, the leaflets are elliptic, dark green in colour and pale on the under surface; variable in size, but often rounded-elliptic, seldom as much as 2.5 cm long. The flowers are generally white and fragrant in large panicles, and produced throughout the year. In Indian tradition *M.oleifera* used for treatment of venomous bites, ascites and rheumatism, helps in lowering blood pressure. Its roots and flower have properties treatment of Cholera and also its root and bark of young tree are rubefacient, stomachic carminative, vescicant and abortifacient. The leaves contain strong antibacterial and antimalarial properties.

3.4: Azadirachata indica

Classification:

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermmatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Rosidae
Order : Sapindales
Family : Meliaceae
Genus : *Azadirachata*
Species : *indica*

Fig 9: *Azadirachata indica*

Characteristics:

A. indica is a highly medicinal herbal plant. Its all parts are effect against antimicrobial activity. It is a fast growing plant with height reach of 15–20 metres (49–66 ft), rarely to 35–40 metres (115–130 ft). It is a evergreen which branches are wide spread. The leaves are pinnate and opposite with length 20–40 centimetres (7.9–16 in) long, dark green colour leaflets about 3–8 centimetres (1.2–3.1 in) long. The flowers are axillary, more-or-less drooping panicles which are up to 25 centimetres (9.8 in) long. The fruits are smooth, globular, which varies in shape from elongate oval to nearly roundish. The neem products are believed as antifungal, antibacterial, antiviral, ant diabetic, sedative. In Ayurveda neem is a herbal component.

3.5: Acorus calamus

Classification:

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta
Division : Magnoliophyta
Class : Liliopsida
Subclass : Arecidae
Order : Arales
Family : Acoraceae
Genus : *Acorus*
Species : *calamus*

Fig 10: *Acorus calamus* (rhizome)

Characteristics:

Acorus is a well known sweet flag. Their leaves are prominent leaf veins with a single prominent midvein and then on both sides slightly raised secondary veins. Its leaves size range 0.7 and 1.7 cm wide, with average of 1 cm. The flower of this plants are too longer with length between 3 and 4 mm with an an abortive ovary with a shrivelled appearance. It grows in clay soils with slightly acidic or alkaline nature and it also grow in water condition. The root is excellence for emmenagogue, aphrodisiac, stimulant, carminative, diaphoretic, hypotensive, expectorant, and febrifuge, aromatic, hallucinogenic, analgesic, sedative, stomachic and vermifuge. Their tonic is a excellent tonic for powers of stimulating and stabilizing the appetite and also treatment for digestive disorders, bronchitis, sinusitis etc. It has a peculiar quality that chewing its root kills the taste for tobacco.

4. OBJECTIVES

4.1: Preparation of plant extract from extracts *(Hibiscus rosa sinensis, Moringa oleifera, Acorus calamus, Cucurbita maxima, Azadirachta indica)*

4.2: Synthesis of silver nanoparticles from five plant extracts.

4.3: Characterization of silver nanoparticles by UV-vis spectroscopy, Scanning electron microscopy, Atomic force microscopy, Dynamic light scattering and zeta potential studies, X-Ray diffraction, Fourier Transmission infrared spectroscopy

4.4: Antimicrobial activity Study against different five clinical pathogens Bacterial samples- *staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Vibrio cholera* and *Escherichia coli*

5. MATERIALS AND METHODS

5.1: Material Required:

5.1.: Plant Samples:

- 5 Plant Samples
    - *Moringa oleifera* leaves
    - *Cucurbita maxima* petal
    - *Azadirachta indica* leaves
    - *Hibiscus rosa sinensis* petal
    - *Acorus calamus* rhizome

5.2: Chemical regent required:

- Silver nitrate ($AgNO_3$)
- Potassium Bromide (KBr)
- Nutrient agar
- Nutrient broth

5.3: Instrument required:

- UV-visible light Spectroscopy (PerkinElmer spectrophotometer)
- SEM (JEOL Jsm-6480 LV)
- FTIR (Shimadzu)
- XRD (x'pert pananalytical)
- DLS (Malvern, UK)
- AFM (Veeco)

5.4: Methods:

5.4: Preparation of plant extract:

- Healthy plant samples were collected from the locality of Rourkela and were cleaned properly in running tap water.
- The samples were shade dried and homogenised to fine powder using a mortar and pestle.

- The solution was then kept at room temperature to cool down.
- The plant extract was then filtered out.

5.5: Synthesis of Silver Nanoparticle:

- The plant extract was then mixed properly.
- The extract solution was then heated.
- The extract solution was then subjected to centrifugation.
- The pellets obtained were washed.
- The pellet obtained was then lyophilized.

5.6: Characterization Techniques:

5.6.1: UV-vis spectra analysis:

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

5.6.2: SEM analysis:

The Morphological characterization of the samples was done using JEOL Jsm-6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto finecoater. After that the material was subjected to analysis.

5.6.3: AFM analysis:

The surface morphology of the nanoparticles were visualised by Atomic force microscope (Veeco) under normal atmospheric conditions. The examined samples were dispersed on small slide and explored on contact mode of the instrument.

5.6.4: DLS Particle size and zeta potential analysis:

The size distribution or average size of the synthesized AgNPs were determined by dynamic light scattering (DLS) and zeta potential measurements were carried out using DLS (Malvern, UK). For DLS analysis the samples were diluted 10 folds using 0.15M PBS (pH 7.4) and the measurements were taken in the range between 0.1 and 10,000 nm.

5.6.5: FT-IR analysis:

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}. The sample were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr , compressed to form an almost transparent disc. KBr was used as a standard analyse the samples.

5.6.6: XRD analysis:

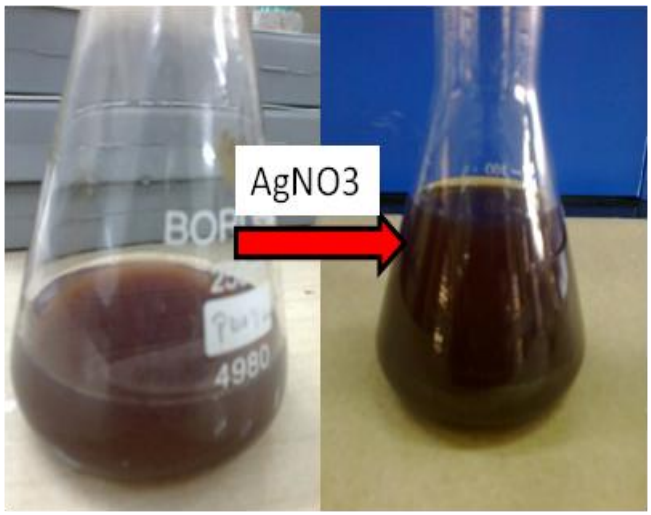
XRD measurements of the reduced AgNPs perform were recorded on X-ray diffractometer (x'pert pananalytical) instrument operating at a voltage of 40 kV and current of 30 mA with Cu K (α) radiation to determine the crystalline phase and material identification. The samples were taken in lids and put under instrument for analysis.

5.6.7: Anti microbial activity:

The clinical pathogenic strains of *Escherichia coli*, *Pseudomonas*, *VIbrio Cholerae*, *Staphylococcus aureus* and *Klebsiella pneumonia* were used to determine the antibacterial activity of the silver nanoparticles by following the method according to Sondi *et al.* 1ml of suspension of approximately 10 5 CFU/ml density of the microorganisms to be tested were distributed uniformly on agar surface plate and incubated at 28°C (CFU = colony forming units).Silver-free agar plates cultured under the same conditions were used as a control. The plates were incubated under 24 hrs at 37˚c and the numbers of colonies were counted after 24 hrs. The counts on the 10 plates corresponding to a particular sample were averaged. The average values were expressed as Mean± Standard deviations.

6. Results and Discussion

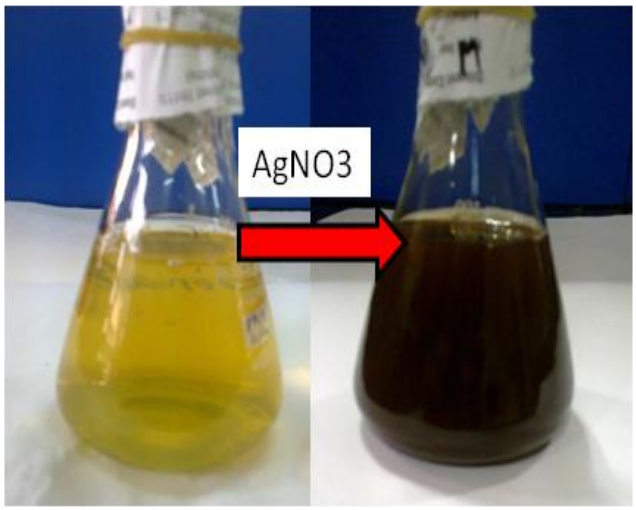
6.1: Visible Observation:



Before After

Hibiscus rosa sinensis

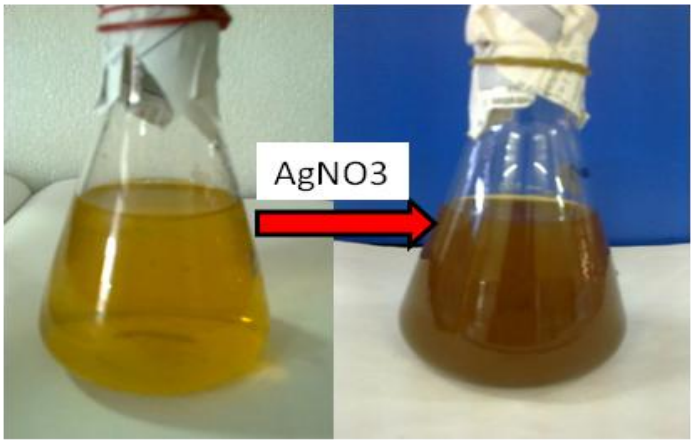
(a)



Before After

Cucurbita maxima

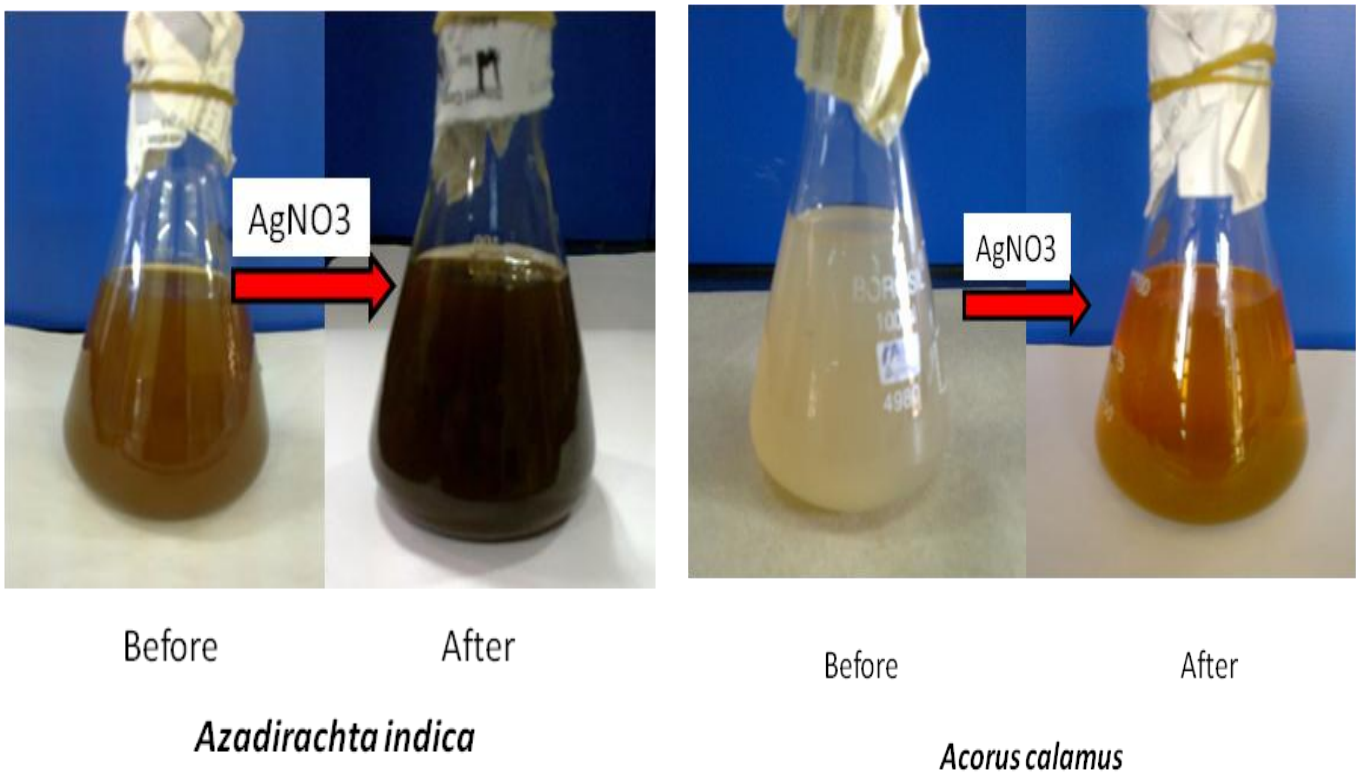
(b)



Before After

Moringa oliefera

(c)

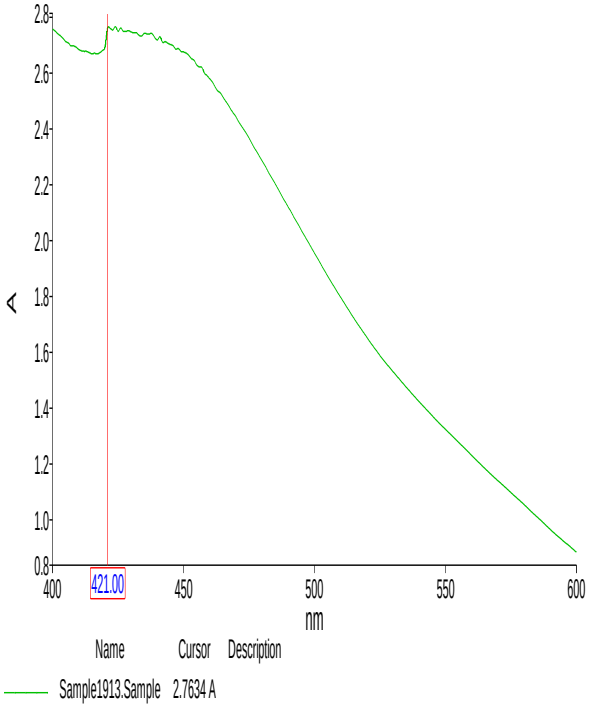


(d) (e)

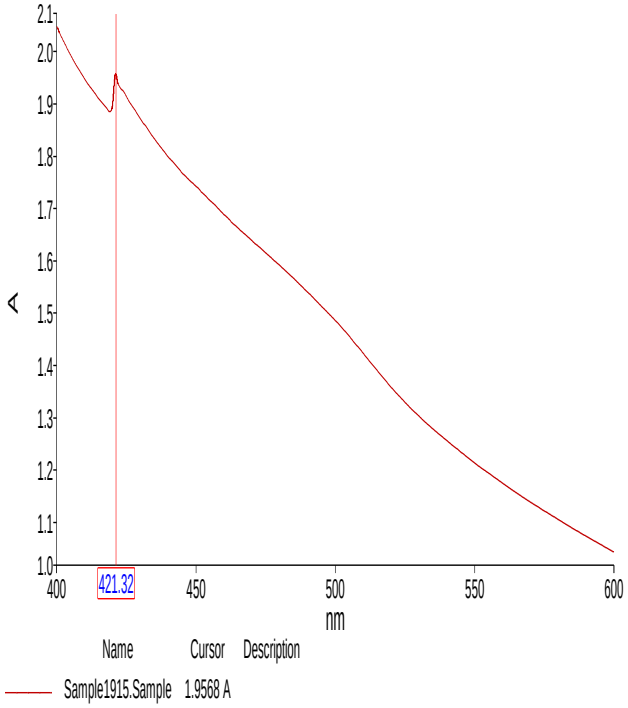
Fig 11: Colour change of plant extract before after addition of $AgNO_3$ (a) *H. sinensis (b) C. maxima (c)M. oliefer*a) (d) *A. indica* (e) *A. calamus*

According to literature studies silver nanoparticle solution has dark brown or dark reddish in colour. In *H.sinensis* before addision of $AgNO_3$ its colour was red but after its treatment with $AgNO_3$ its colour changes to dark brown which indicated the formation of AgNPs. Likewise all the other four plants extact (*H. sinensis, C. Maxima, M. Oliefer*a, *A. Indica, A. calamus*) colour changed to dark brown after treatment with $AgNO_3$.(Fig. 11) This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles.

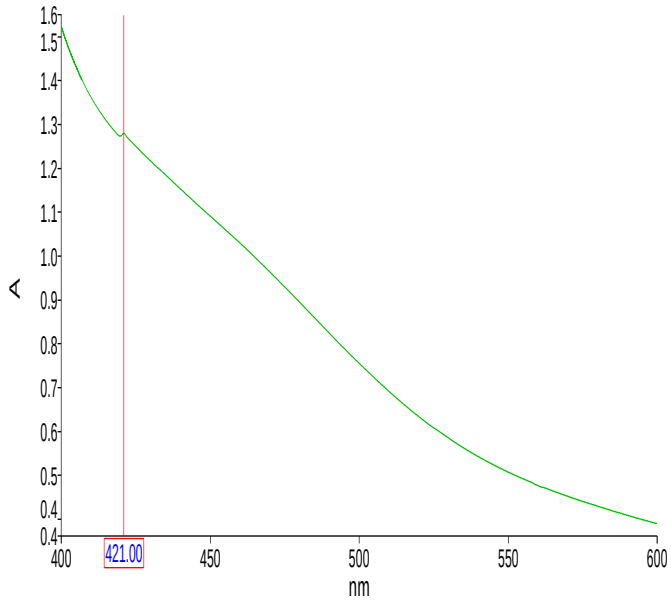
6.2: UV-vis spectroscopy:



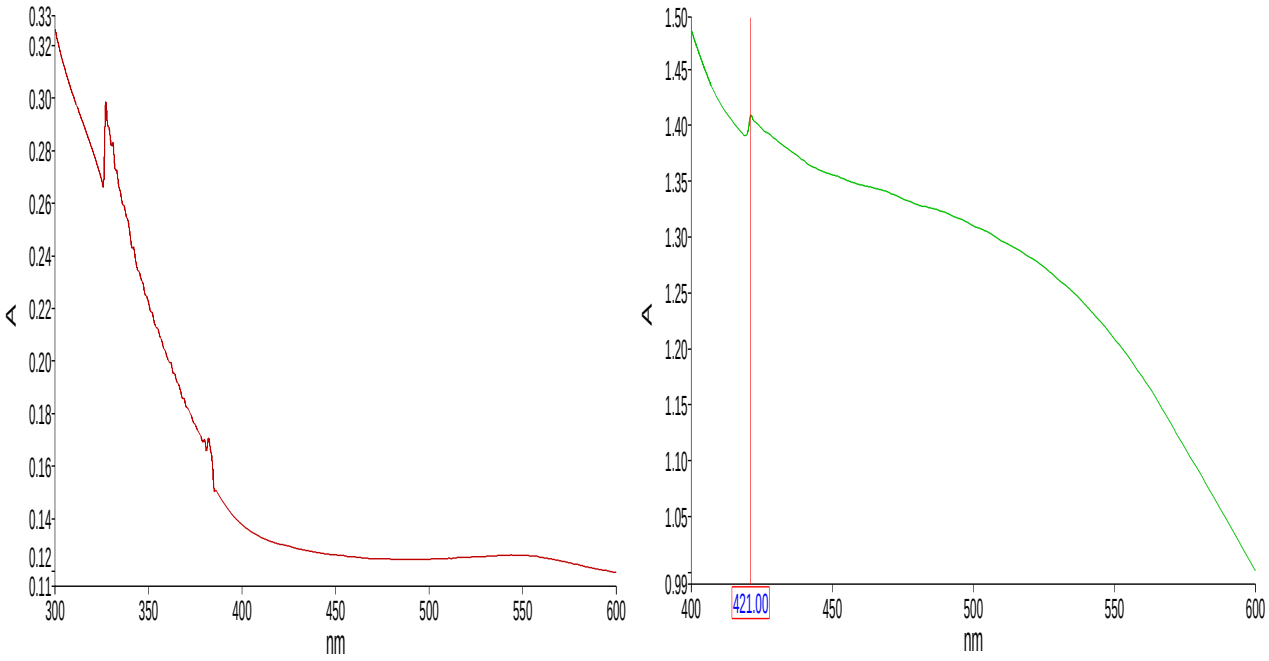
H. sinensis



C. maxima



M. oleifera

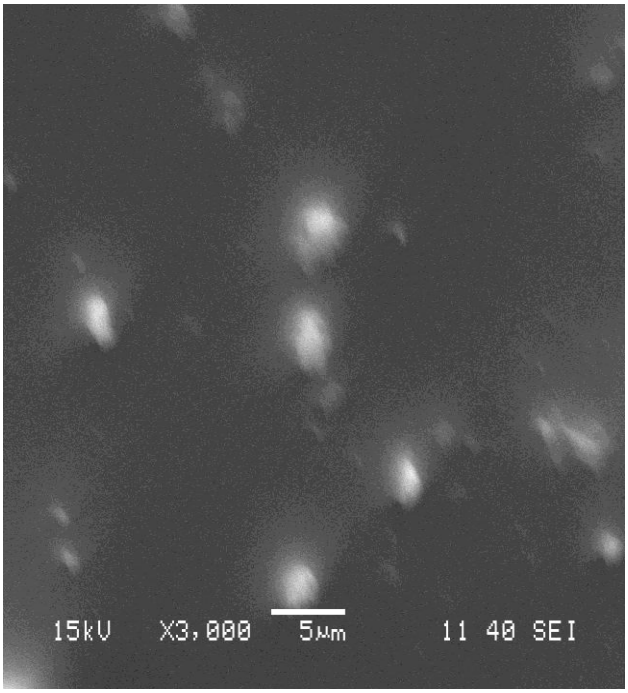


A. indica *A. calamus*

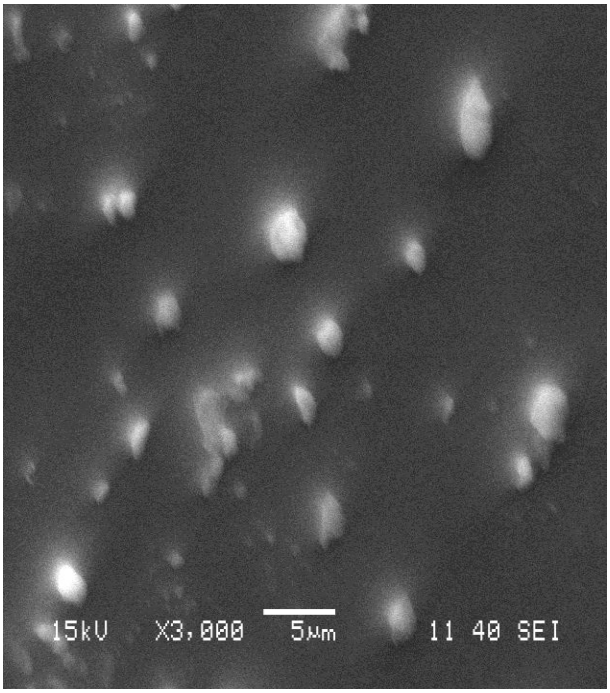
Fig 12: UV-visible absorption peak of *H. sinensis, C. maxima, M .oliefera, A. indica, A. calamus* synthesized silver nanoparticles approximately at 421nm.

The UV absorption peak of silver nanoparticles range from 400 nm – 450 nm (Ramteke *el al.*, 2013). Fig. 12 shows the UV absorption peaks of *H. Sinensis, C. maxima, M. oleifera, A. indica* and *A. calamus*.UV-Vis spectra shows the peaks approximately at 421.00nm, clearly indicating the formation of spherical AgNPs in all the plants extracts. The occurrence of the peak at 421 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field (Naheed *et al.*, 2011).

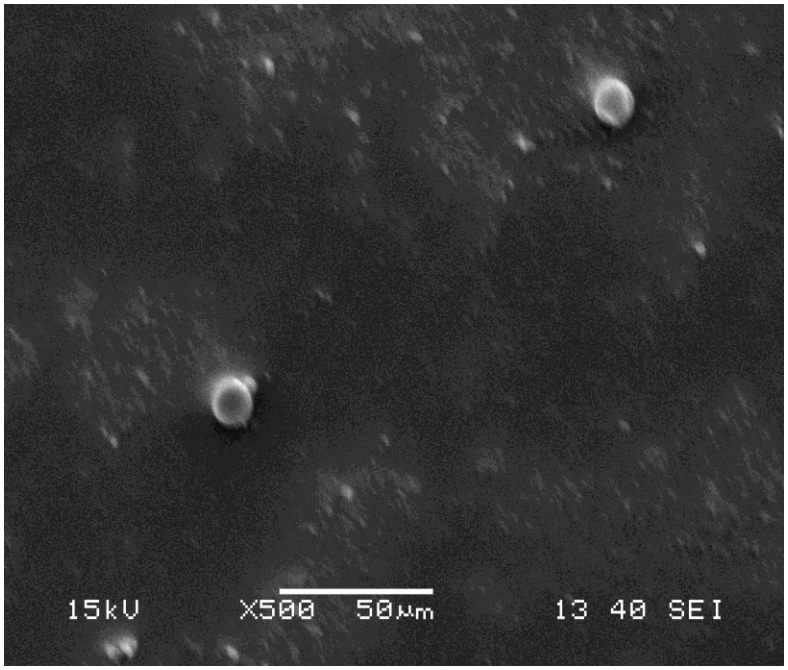
6.3: Scanning Electron Microscopy:



H. sinensis
(a)

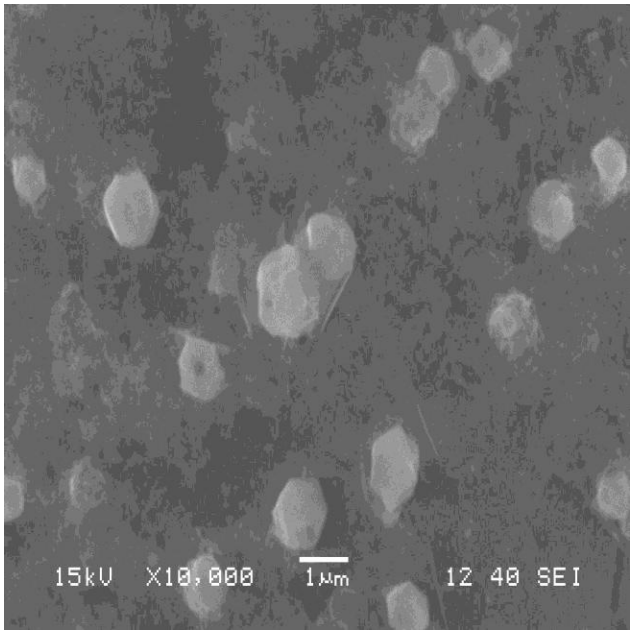


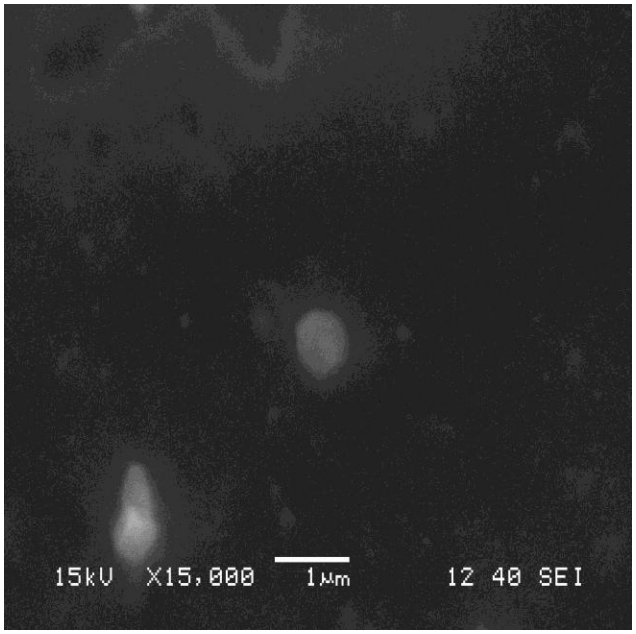
C. maxima
(b)



M. oleifera

(c)





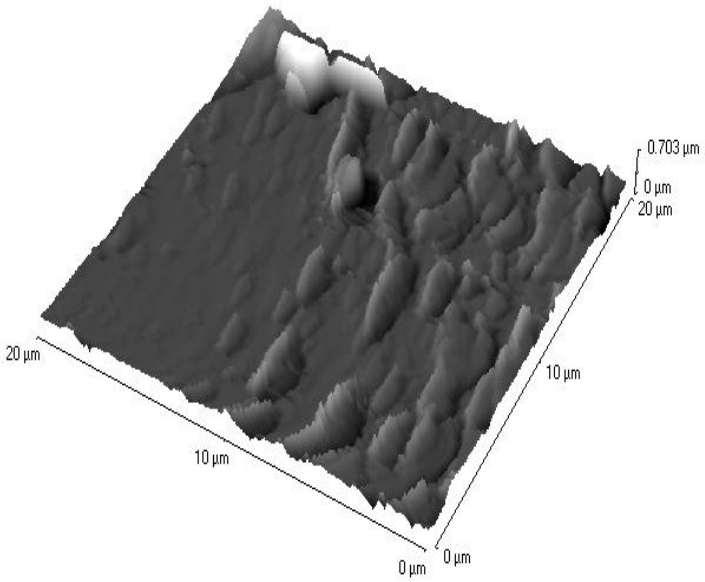
A. indica *A. calamus*

(d) (e)

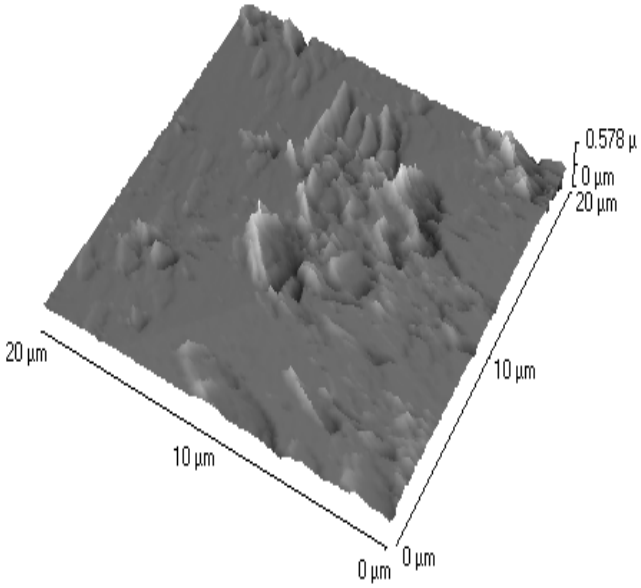
Fig 13: SEM image of (a) *H. sinensis,* (b) *C. maxima*, (c) *M. oliefera* (d) *A. indica*, (e) *A. calamus*

A scanning electron microscope was employed to analyze the shape of the silver nanoparticles that were synthesised by green method. SEM analysis shows that the five plants have tremendous capability to synthesize silver nanoparticles which were roughly spherical in shape (fig.13) and were uniformly distributed.

6.4: AFM Analysis:



(a)



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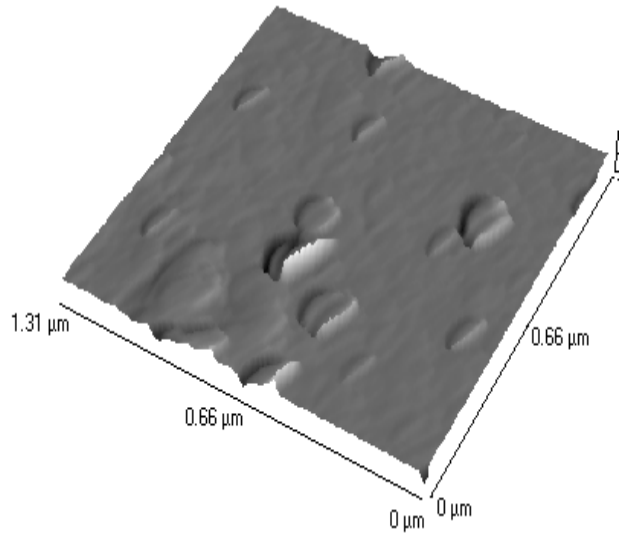

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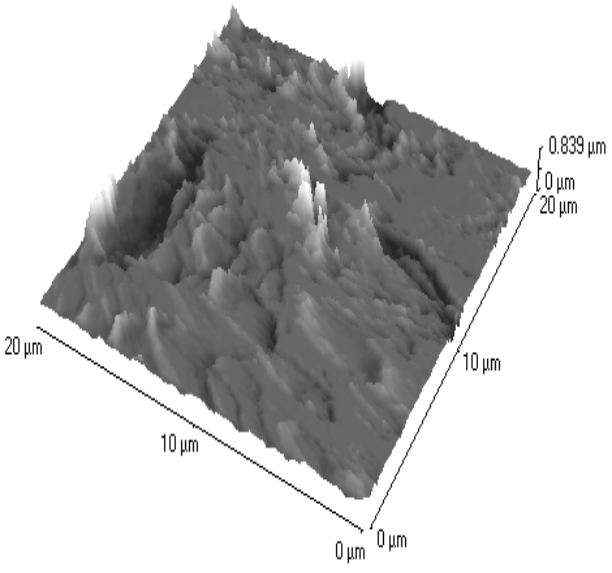
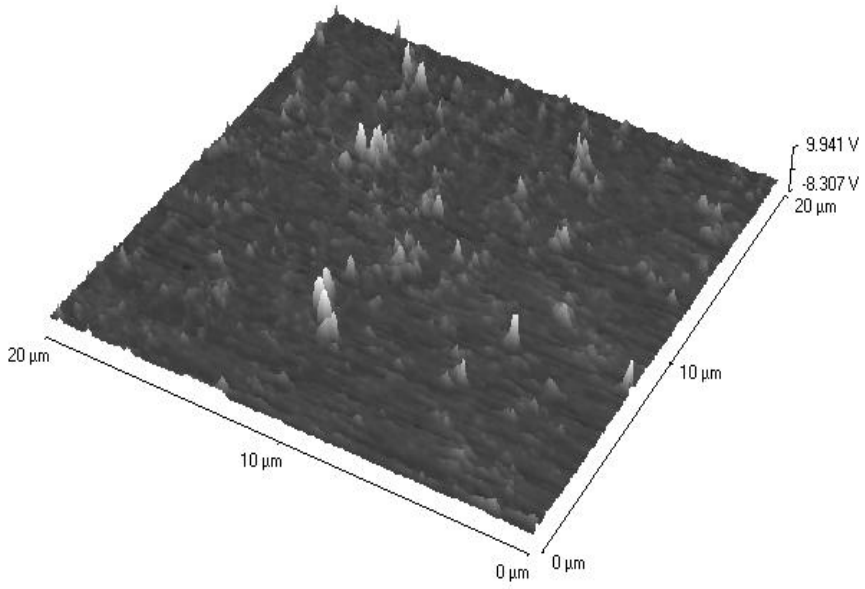


(d)



(e)

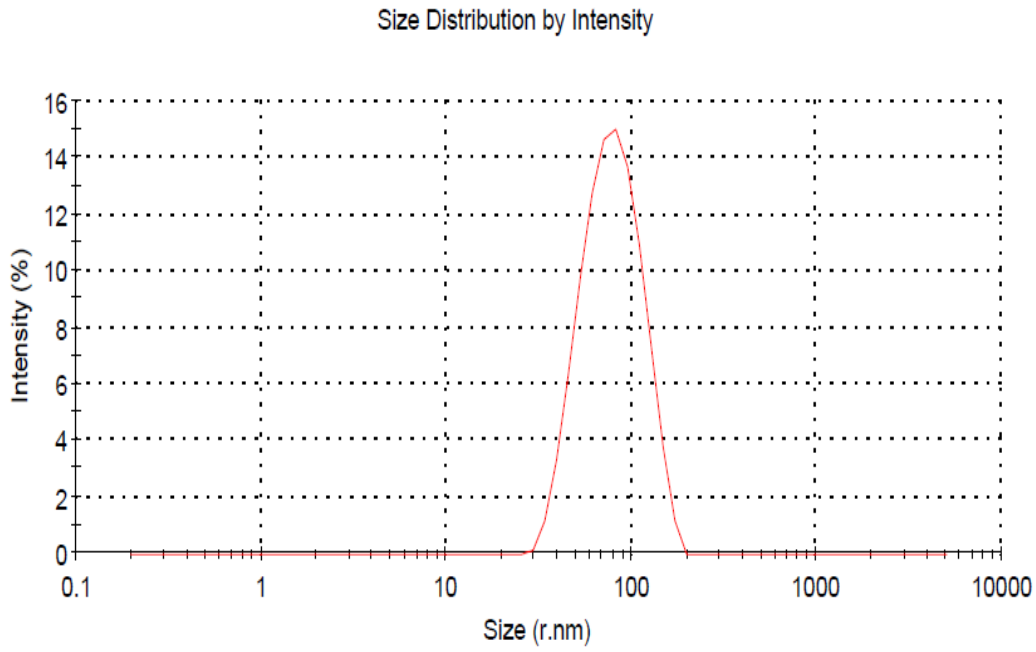
Fig 14: AFM images of (a) *H. sinensis,* (b) *C. maxima,* (c) *M. oliefera* (d) *A. indica,* (e) *A. calamus*

AFM was used to analyse the particle morphology (shape, size). AFM image of *H. Siniesis* mediated synthesised AgNPs shows that they have a uniformly packed surface with height 0.703µm. Fig 14 shows the 3D AFM images of the plant extract mediaited synthesised nanoparticles and size of the particles are shown in table no 2.

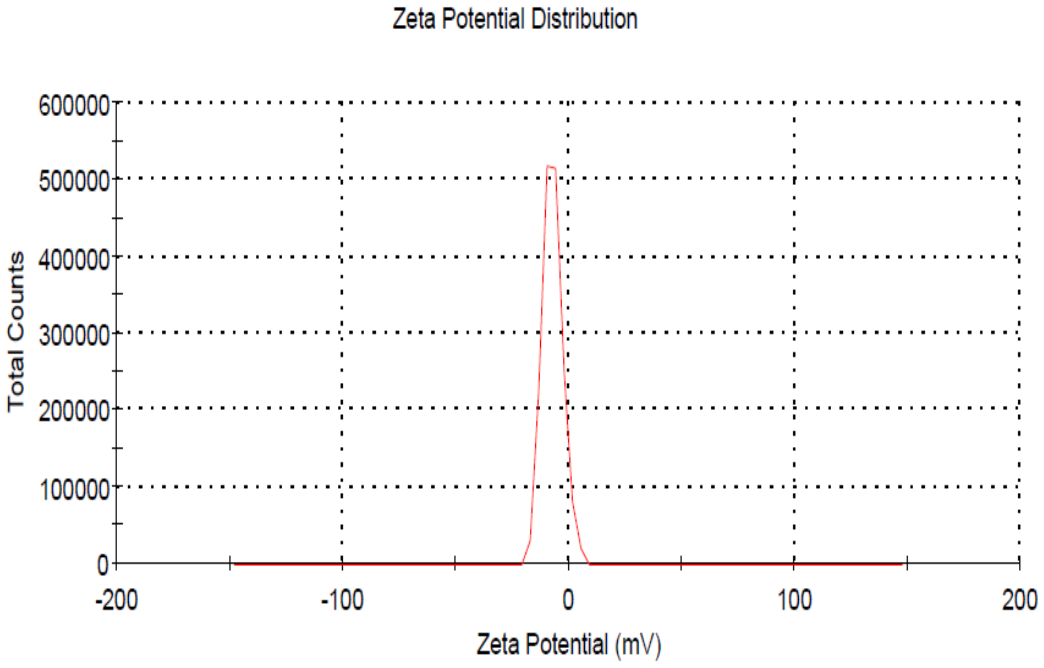
Table 2: Size of the silver nanoparticle synthesized from five different plants.

Sl.No	**Plant Sample**	**Size of AgNPs**
1	*H. sinensis*	0.703µm
2	*C. maxima*	0.578µm
3	*M. oliefera*	3.341µm
4	*A. indinca*	1.023µm
5	*A. calamus*	0.839µm

6.5: DLS and Zeta Potential studies:

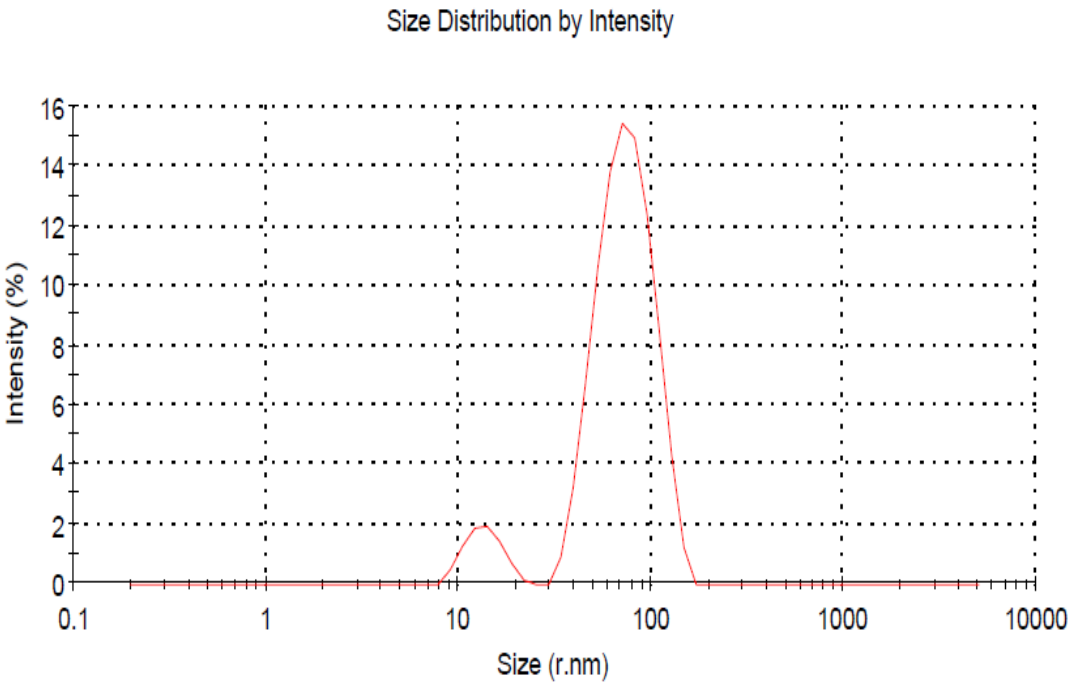


(a)

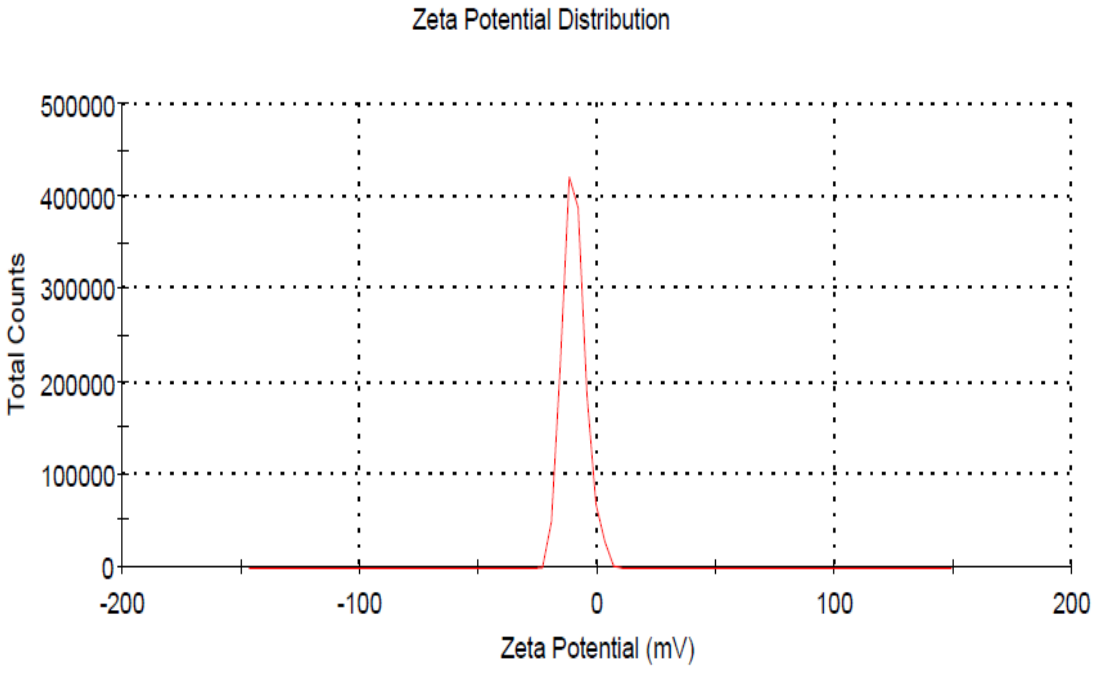


(b)

Fig 15: (a) DLS (b) Zeta potential graph of *H.sinensis* mediated synthesized AgNPs

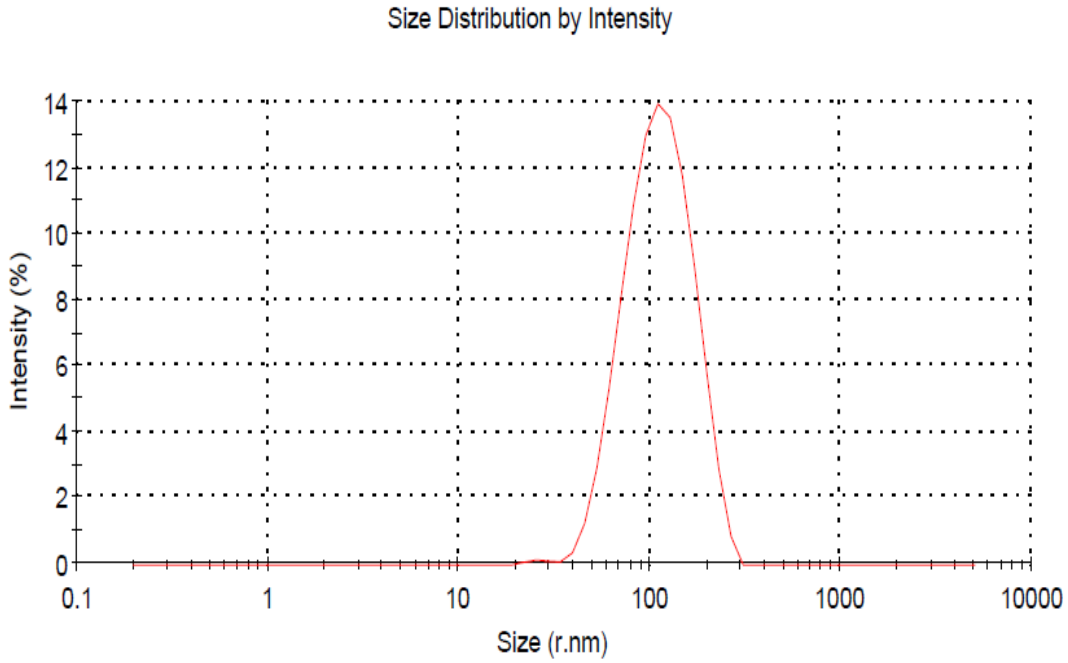


(a)

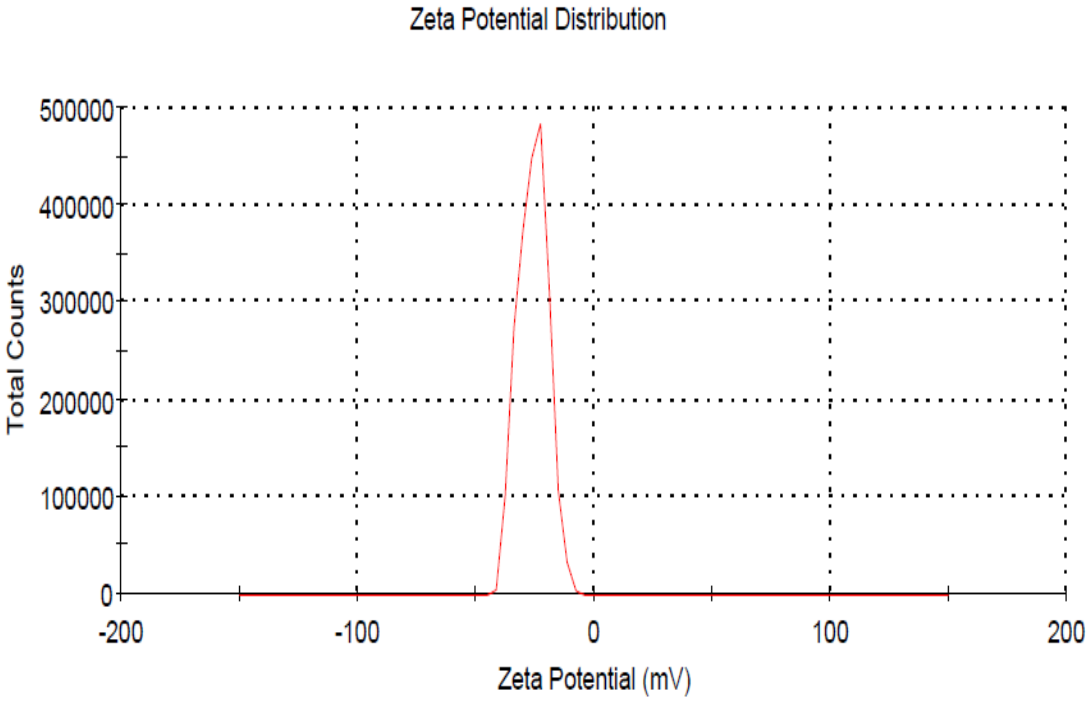


(b)

Fig 16:(a) DLS (b) Zeta potential graph of *C. maxima* mediated synthesized AgNPs

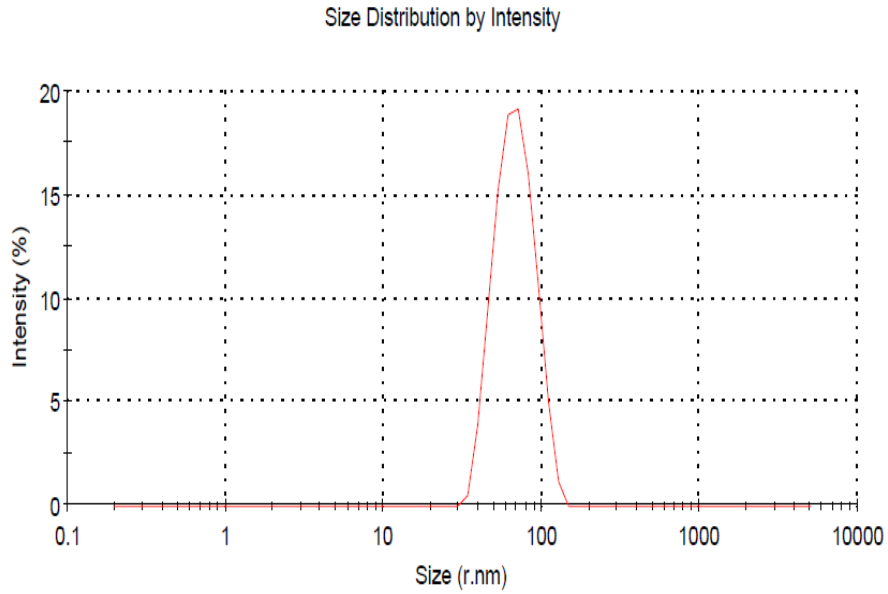


(a)

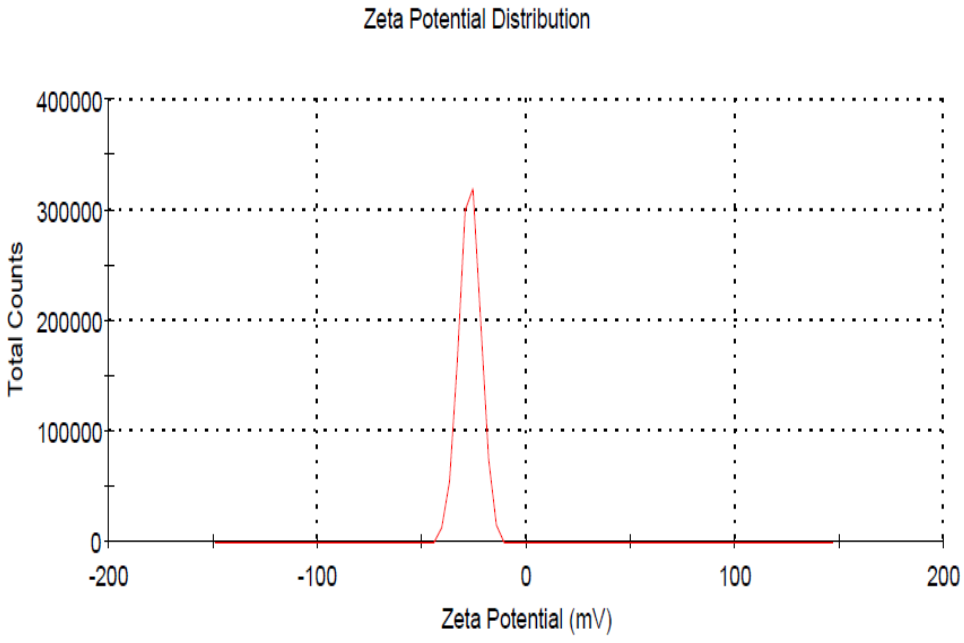


(b)

Fig17 :(a) DLS (b) Zeta potential graph of *M. oliefera* mediated synthesized AgNPs

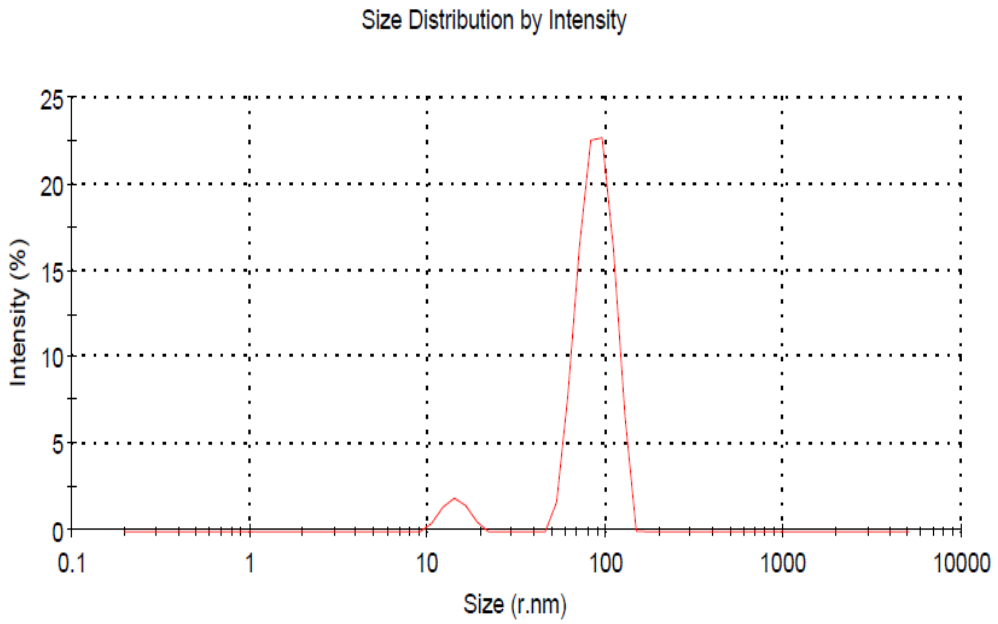


(a)

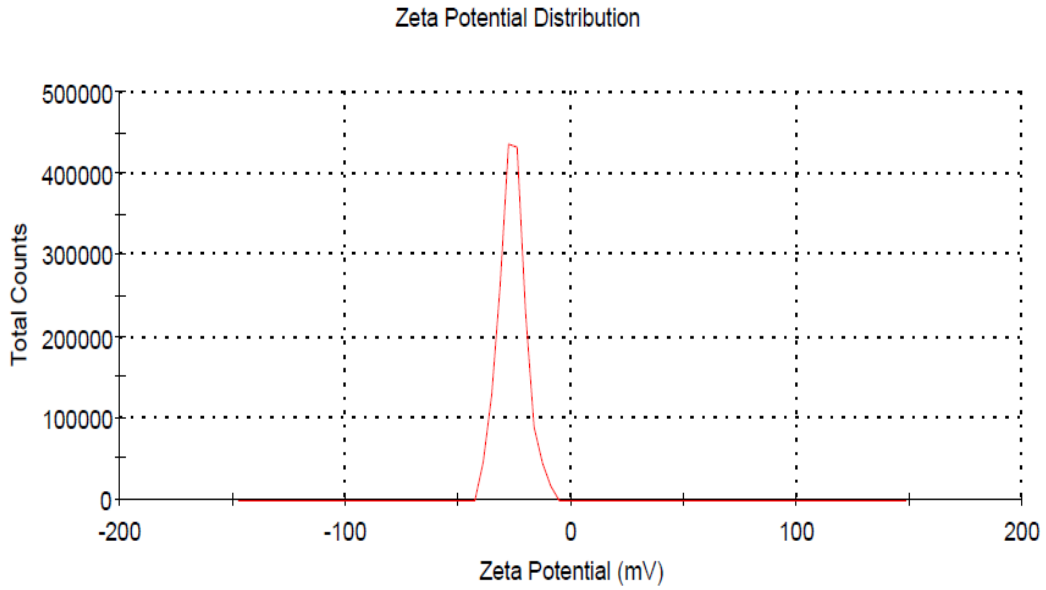


(b)

Fig18:(a) DLS graph (b) Zeta potential graph of *A.indica* mediated synthesized AgNPs



(a)



(b)

Fig19:(a) DLS graph (b) Zeta potential graph of *A.calamus* mediated synthesized AgNPs

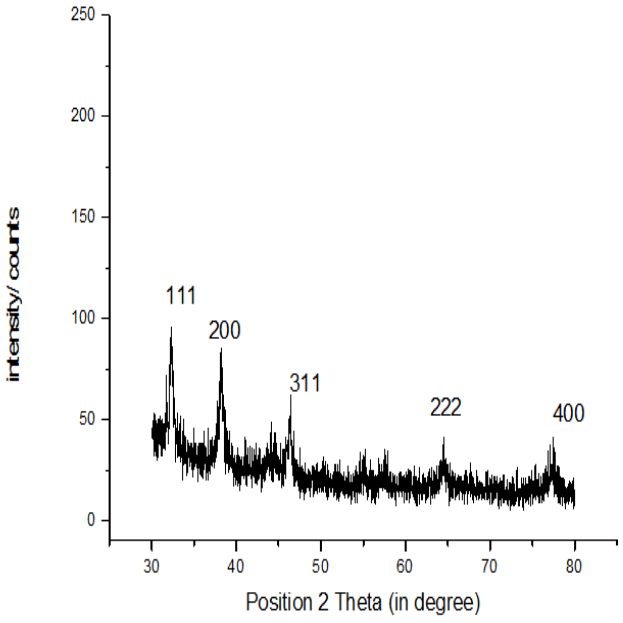
Dynamic light scattering (DLS) is a technique used to determine the size, size distribution profile and poly dispersity index of particles in a colloidal suspension. Fig 15 shows the DLS and zeta potential graph of *H. sinensis* which has an average size of 76.27nm and the particles carry a charge of -7.22 mV. Fig 16 shows the DLS and zeta potential graph of *C. maxima* which has an average size of 76.27nm and the particles carry a charge of-9.81 mV. Fig 17 shows the DLS and zeta potential graph of *M. oleifera* which has an average size of 105.0 nm and the particles carry a charge of -27.1 mV. Fig 18 shows the DLS and zeta potential graph of *A. indica* which has an average size of 124.1 nm and the particles carry a charge of -25.9 mV. Fig 19 shows the DLS and zeta potential graph of *A. calamus* which has an average size of 76.27 nm and the particles carry a charge of -26.1 mV. Poly disparity index (PDI) is a measurement for distribution of silver nanoparticle with from 0.000 to 0.5. PDI greater than 0.5 values indicates the aggregation of particles. From the table 3, it was clear that all the AgNPs synthesised from the five plant extracts does not aggregate at all.

Zeta potential measures the potential stability of the particles in the colloidal suspension. Silver nanoparticles generally carry a negative charge. All silver nanoparticles synthesized from the five plants showed negative charge and were stable at room temperature.

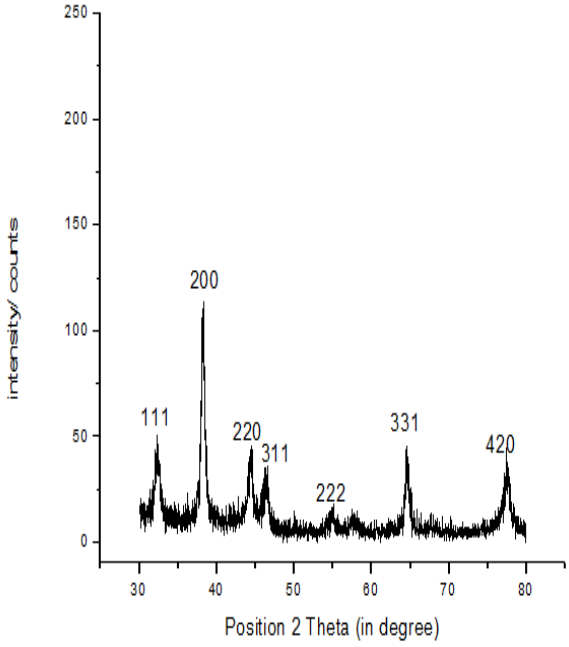
Table 3: Synthesized silver nanoparticle from five plants size, PDI and zetapotential

Sl.No	**Plant samples**	**DLS (size)**	**PDI**	**Zeta potential**
1	*H.sinensis*	76.27 nm	0.405	-7.22 mV
2	*C. maxima*	76.10 nm	0.352	-9.81 mV
3	*M. oleifera*	105.0 nm	0.168	-27.1 mV
4	*A. indica*	124.1 nm	0.307	-25.9 mV
5	*A. calamus*	76.27 nm	0.405	-26.1 mV

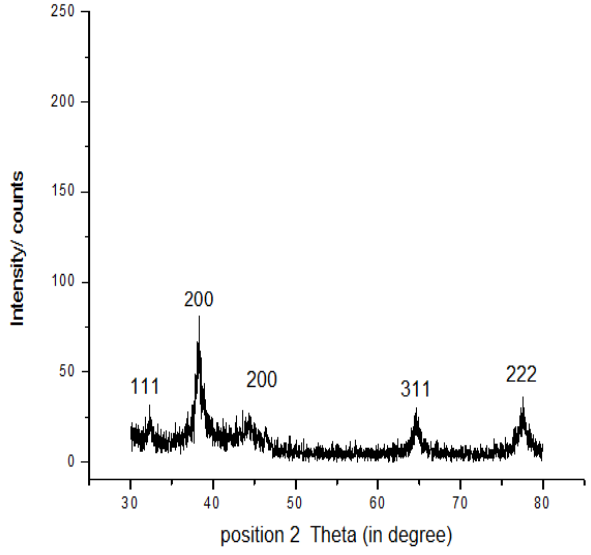
6.6: XRD analysis:



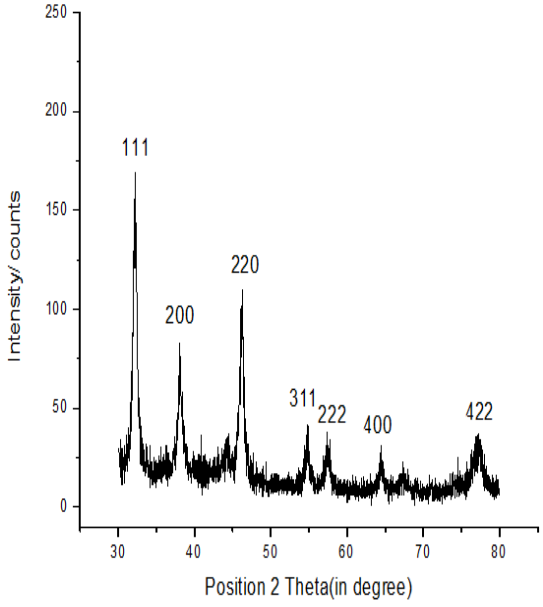
(a)



(b)



(c)



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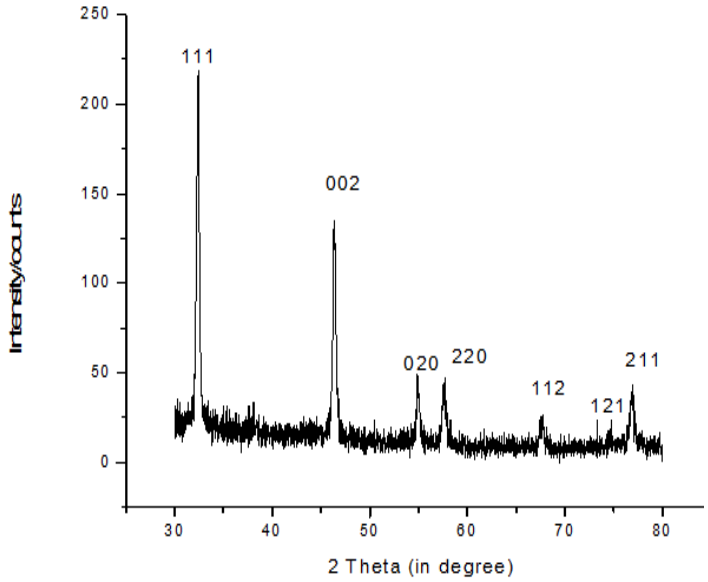
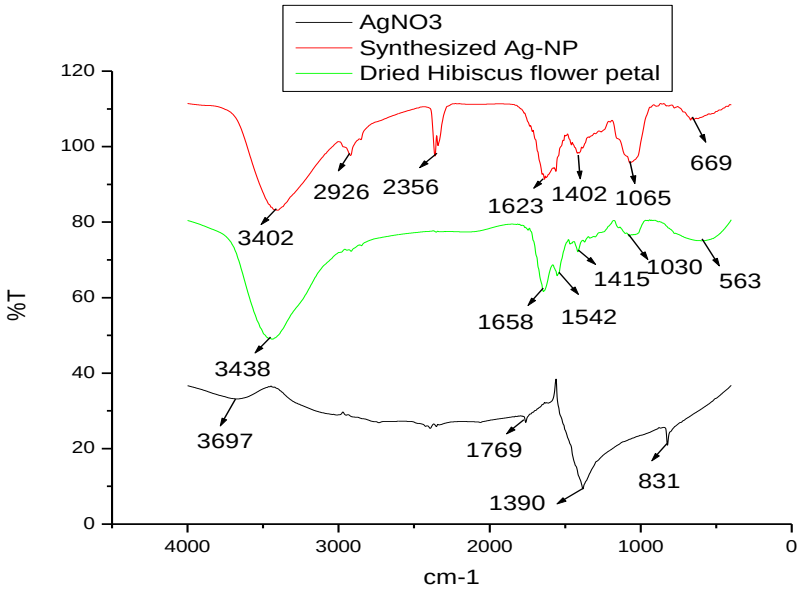


(e)

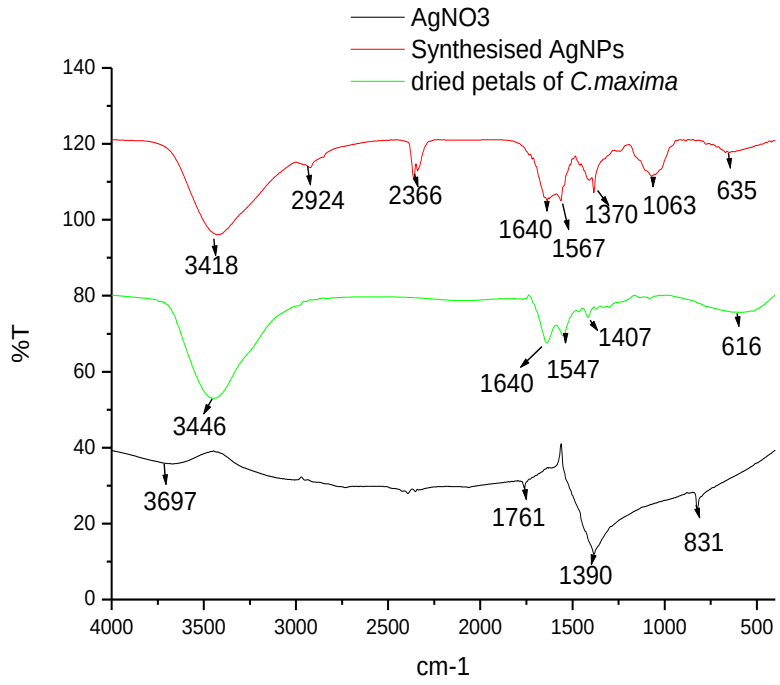
Fig 20: XRD patterns of (a) *H.sinensis*, (b) *C. maxima*, (c) *M. oliefera* (d) *A. indica* (e) *A. calamus*

XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesised nanoparticles particles. Fig 20 shows the XRD patterns of H.*sinensis*, *C. maxima*, *M. oliefera*, *A. indica* and *A. calamus*. With reference to the JCPDS data file No. 04-0783 it was concluded that the nanoparticles were crystalline in nature having cubical shape with no such impurities.

6.7: FTIR Analysis:



(a)



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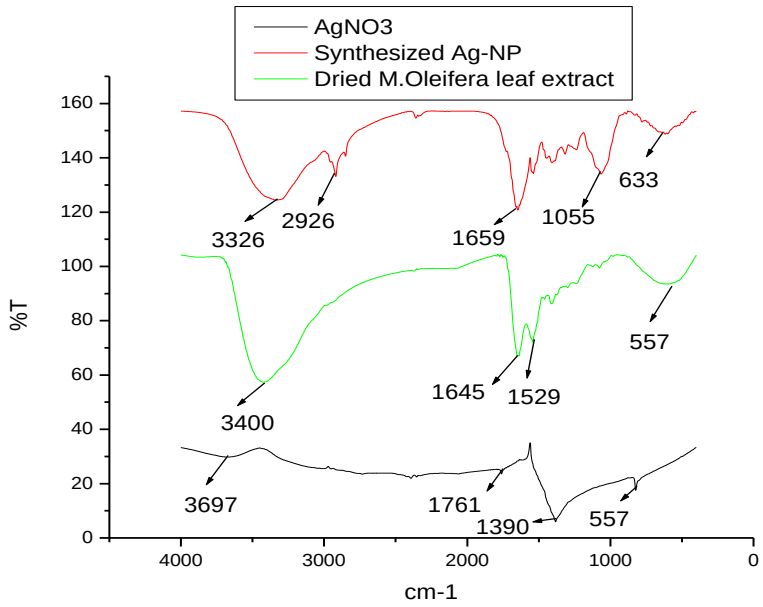
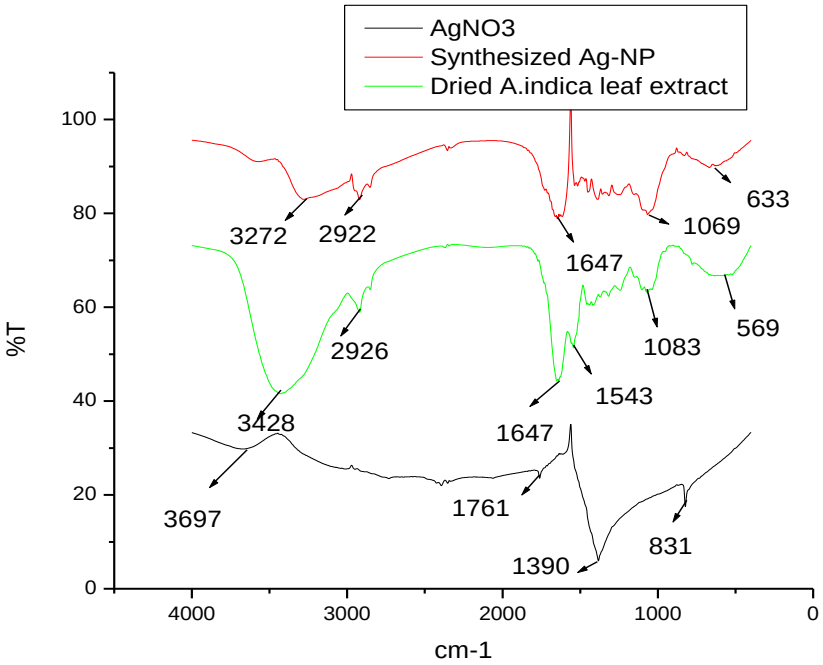

AgNO3
Synthesized Ag-NP
Dried M.Oleifera leaf extract
%T
cm-1
3326
2926
1659
1055
633
3400
1645
1529
557
3697
1761
1390
557

(c)


AgNO3
Synthesized Ag-NP
Dried A.indica leaf extract
%T
cm-1
3272
2922
1647
1069
633
2926
1083
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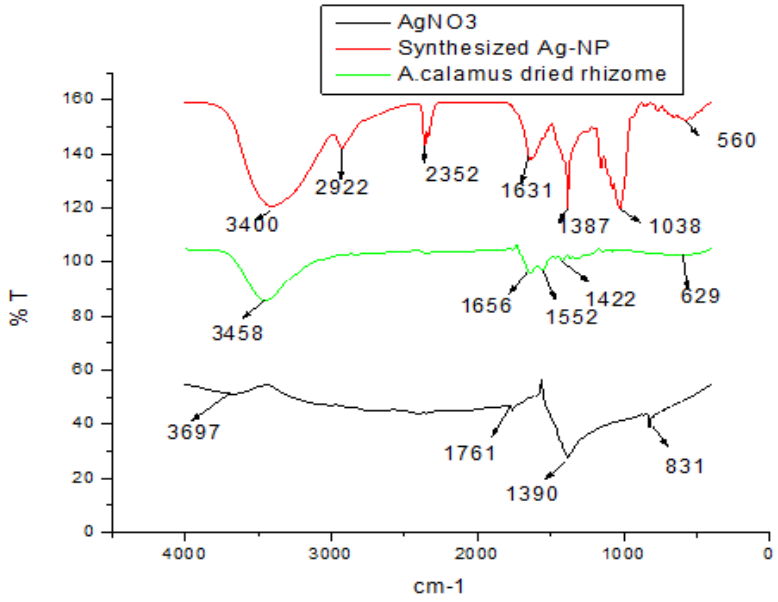
3428
1543
1647
3697
1761
1390
831

(d)



(e)

Fig 21: FTIR graph of (a) *H.sinensis,* (b) *C. maxima*, (c) *M. oliefera* (d) *A. indica* (e) *A. calamus*

FTIR gives the information about functional groups present in the synthesised silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the plant extracts. Fig 21 (a) shows the FTIR spectrum of *H. sinensis* mediaited synthesised AgNPs, the silver nitrate salt and dried *H.sinensis* petal extract, in $AgNO_3$ peaks were observed at 3697cm^{-1},1761cm^{-1},1390cm^{-1},831cm^{-1}which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. In the *H. sinensis petal* extracts peak were observed at 3407.70cm-1, 2919.19 cm-1, 2360.78 cm-1, 2340.92 cm-1, 1639.62 cm-1, 1419.77 cm^{-1}, 1071.77 cm^{-1}, 1071.46 cm^{-1}, 669.75 cm^{-1} which are associated OH stretching, CH stretching, C=N stretching, C=N stretching, N-H stretching, CH stretching, CN stretching, C-Cl stretching. In the synthesized AgNPs from*H.sinensis* peaks were observed at 3439.72cm^{-1}, 1644.15cm^{-1}, 1553.96 cm^{-1}, 1416.58 cm^{-1}, 1076.07 cm^{-1}, 597.71 cm^{-1} which are associated with NH stretching, C=O stretching, N-O stretching, CH_2 & CH_3 deformation, C-O stretching and halogen group

presence. The *H. sinensis* plant extract shows broad peak at 3407.70cm^{-1} which indicate the presence of OH group or carboxyl groups and after synthesis of AgNPs there is a shift in the broad peak to the right at 3439.72cm-^{1} indicating the NH stretching. These carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the biofabrication of the nanoparticles by the action of the protein or phytochemicals. Fig (b) to (e) clearly illustrates the biofabrication of the AgNPs by the action of the phytochemicals such as phenols, terpenoids, flavonoids and alkaloids in C. *maxima, M. oliefera, A. indica* and *A. calamus* (Kong and Yu, 2007).

6.8: Antimicrobial Activity:

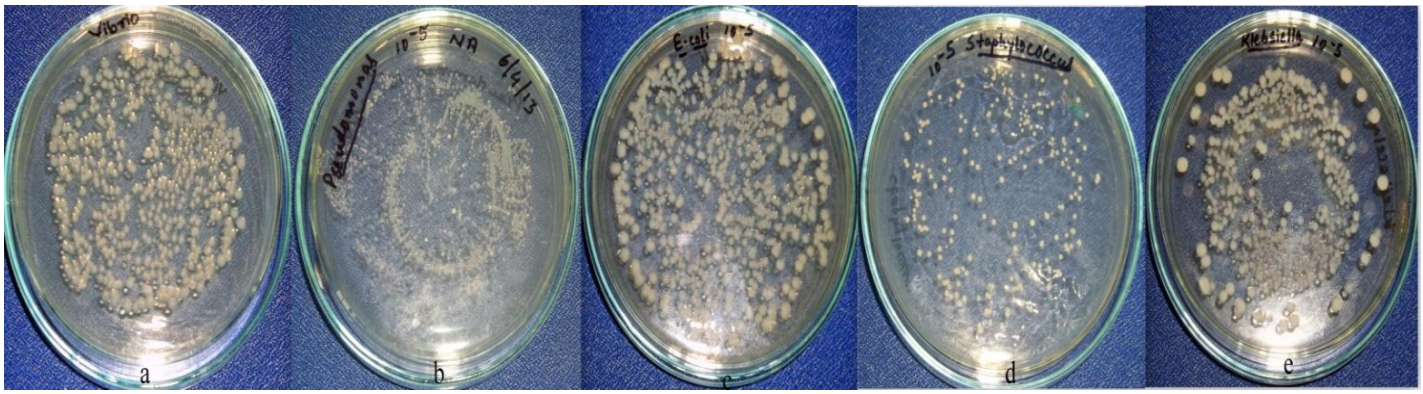


Fig 22: Control plates of five pathogen (a) *Vibrio cholerae aureus* (b) *Pseudomonas aeruginosa,* (c) *Escherichia coli* (d) *staphylococcus aureus* (e) *Klebsiella pneumoniae*

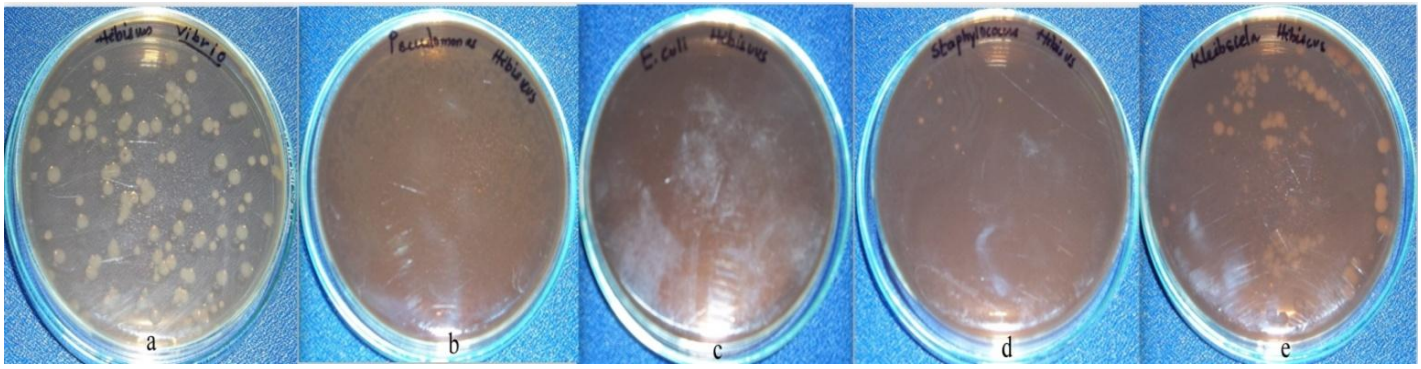


Fig 23: 100µg/ml *H.sinensis* mediaited synthesisied AgNP against (a) *Vibrio cholerae* (b) *Pseudomonas aeruginosa,* (c) *Escherichia coli* (d) *staphylococcus aureus* (e) *Klebsiella pneumoniae*

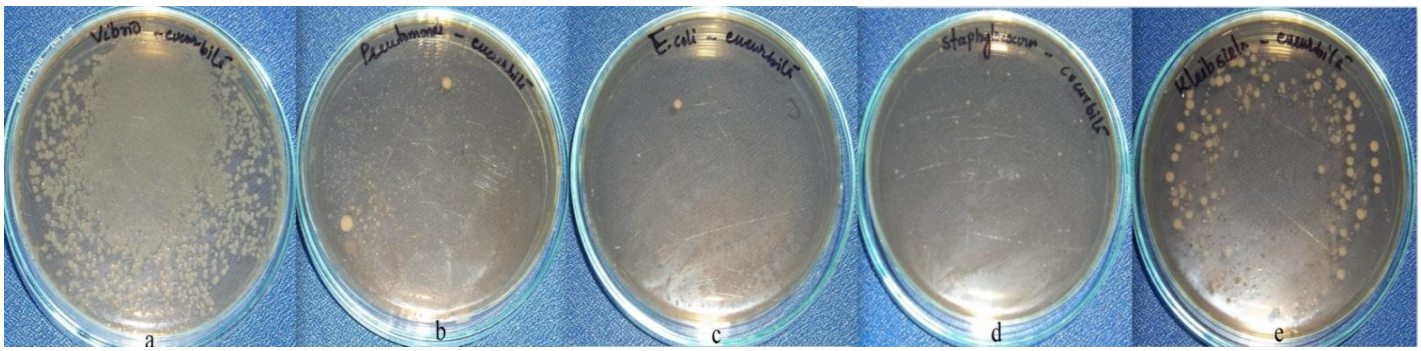


Fig 24: 100µg/ml *C.maxima* mediated synthesized AgNPs against (a) *Vibrio cholerae* (b) *Pseudomonas aeruginosa,* (c) *Escherichia coli* (d) *staphylococcus aureus* (e) *Klebsiella pneumoniae*

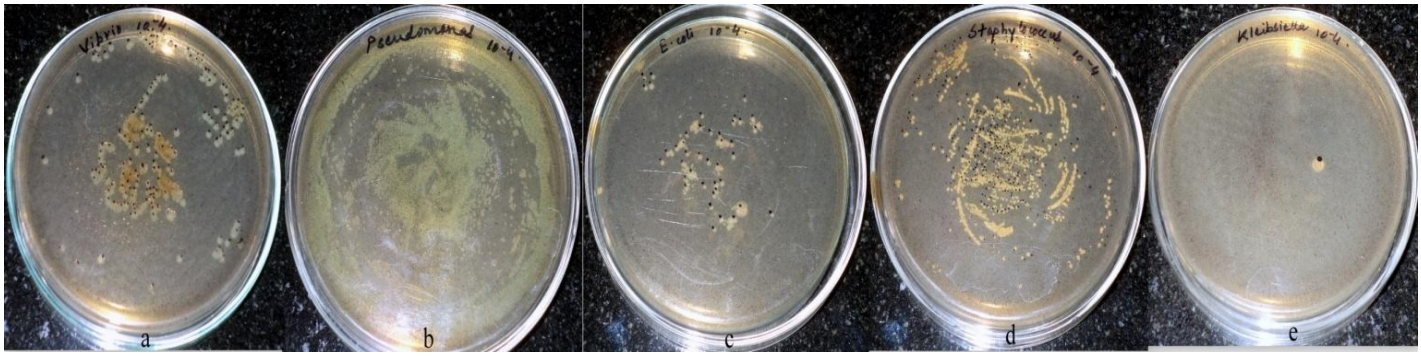


Fig 25: 100µg/ml *M.oleifera* mediaited synthesized AgNPs against (a) *Vibrio cholerae* (b) *Pseudomonas aeruginosa,* (c) *Escherichia coli* (d) *staphylococcus aureus* (e) *Klebsiella pneumoniae*

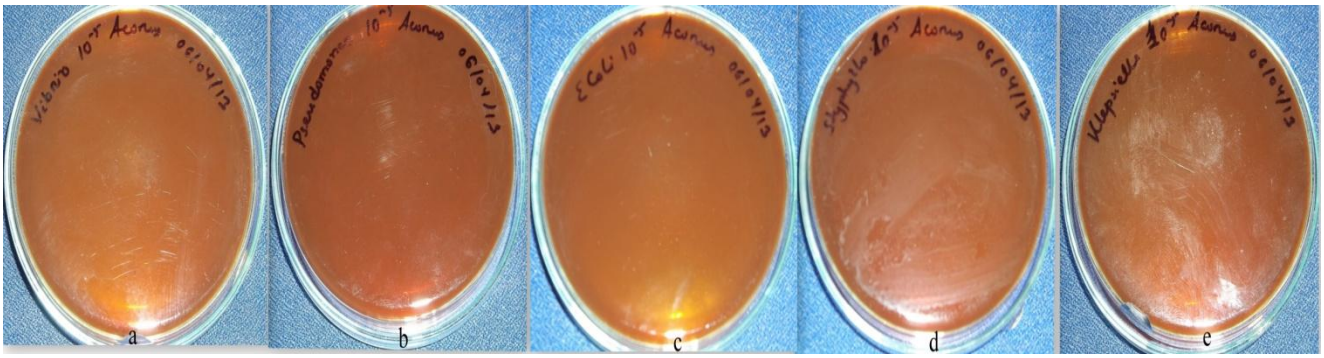


Fig 26: 100µg/ml *A.calamus* mediated synthesized AgNPs against (a) *Vibrio cholerae* (b) *Pseudomonas aeruginosa,* (c) *Escherichia coli* (d) *staphylococcus aureus* (e) *Klebsiella pneumonia*

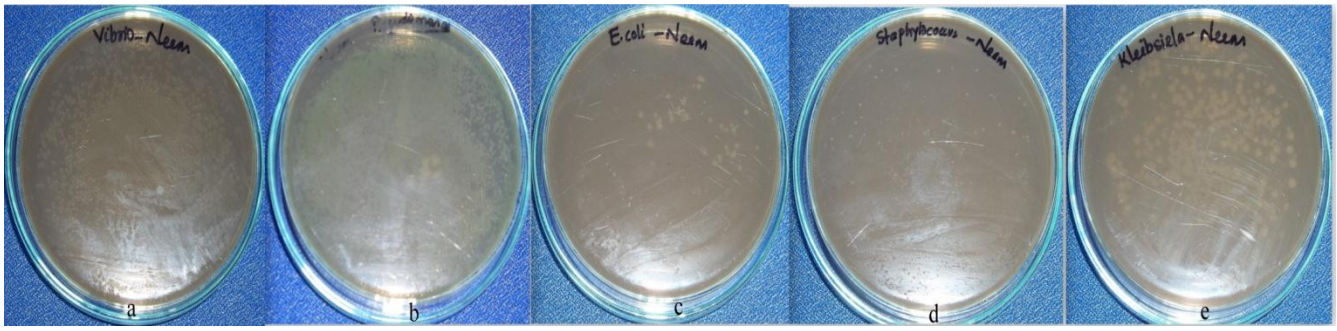


Fig 27: 100µg/ml *A.indica* mediated synthesized AgNPs against (a) *Vibrio cholerae* (b) *Pseudomonas aeruginosa,* (c) *Escherichia coli* (d) *staphylococcus aureus* (e) *Klebsiella pneumoniae*

Antimicrobial activity of the synthesized AgNPs from the five plant extracts testing was done against five clinically important pathogens *Vibrio cholerae aureus, Pseudomonas aeruginosa, Escherichia coli, staphylococcus aureus and Klebsiella pneumonia* by following the procedure of sondi *et al.* which showed promising antibacterial activity against all the pathogens except P. *aeruginosa* .The exact mechanism behind the antimicrobial activity of nanoparticles is not clearly known but some of the hypotheses provided are:

- Attachment to the bacterial cell wall and changing the permeability of the cell membrane.
- Production of Reactive oxygen species and damage the cell membrane.
- Bind to DNA and leads to problem in DNA replication.

Table 4: Antimicrobial activity against five clinically important pathogens.

Sl. No	**Plant Sample**	***staphylococcus aureus***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***	***Vibrio cholerae***	***Escherichia coli***
1	*H.sinensis*	++	+	++	++	++
2	*C. maxima*	++	+	+	++	++
3	*M. oleifera*	+	+	++	++	++
4	*A. indica*	+	++	+	+	++
5	*A. calamus*	++	++	+	++	++

++ = good antimicrobial activity

+ = considerably lower antimicrobial activity

7. Conclusion

Green synthesis of silver nanoparticles by the help of green plants is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a large scale. *H. sinensis, C. maxima,M. oleifera,A. indica and A.calamus* showed great capability to synthesis AgNPs at optimum temperature conditions. The UV absorption peak at 421nm clearly indicates the synthesis of AgNPs. The SEM and AFM studies were helpful at deciphering their morphology and distribution. DLS and Zeta potential studies validated the size and charge of the nanoparticles in the colloidal system without any aggregation. FTIR studies confirmed the biofabrication of the AgNPs by the action of different phytochemicals with its different functional groups present in the extract solution. The XRD patterns confirmed the purity, phase composition and nature of the synthesised nanoparticles. The AgNPs have great antimicrobial activity against K. *pneumonia*, *S*.aureus, *V. cholerae* and *E.coli.* By comparing the different characteristic data we could conclude that AgNPs synthesised from *H. sinensis* and *C. maxima* were less stable then the other three plant extracts which could be due to the absence of capping and stabilizing materials as petals mainly contains pigments for attracting different insects for pollination.

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