A DISSERTATION ON

Biosynthesis of Gold Nanoparticles by using Kalanchoe pinnata aqueous leaf extract as a reducing and capping agent

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Saima Parveen**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled ***"Biosynthesis of Gold Nanoparticles by using Kalanchoe pinnata aqueous leaf extract as a reducing and capping agent"*** successfully. She has completed this work from 2 Feb to 2 June 2022 at the Department of Biosciences, Integral University, under the guidance of **Dr. Salman Khan**.

The dissertation was a compulsory part of her M.Sc. degree. I wish her good luck and a bright future.

(**Dr. Snober S. Mir**)

Head,

Department of Biosciences,

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Saima Parveen**, during the months 2 Feb to 2 June 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the script of the thesis has been written by the candidate herself. The thesis entitled ***"Biosynthesis of Gold Nanoparticles by using Kalanchoe pinnata aqueous leaf extract as a reducing and capping agent"*** is, therefore, being forwarded for acceptance in partial fulfillment of the requirements for the degree award of the student of M.Sc. Biotechnology (IV semester), Department of Biosciences, Integral University, Lucknow, (U.P).

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Saima Parveen **Date**

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LIST OF ABBREVIATIONS

nm	Nanometer
AuNPs	Gold Nanoparticles
DNA	Deoxyribonucleic Acid
QDs	Quantum Dots
MRI	Magnetic Resonance Imaging
TON	Turn Over Number
TOF	Turn Over Frequency
SPR	Surface Plasmon Resonance
IR	Infra-red
TEM	Transmission Electron Microscopy
DLS	Dynamic Light Scattering
PBS	Phosphate Buffer Saline
MHA	Mueller Hinton Agar
CNT	Carbon Nanotubes

Introduction

Nanotechnology

Human observation and imagination more frequently would be the cause of invitation to new science and technology. Nanotechnology, a 21st-century frontier, was the basic reason for such dreams. Nanotechnology is defined as the understanding and control of matter at dimensions between 1 and 100 nm where unique phenomena enable novel applications. Although human exposure to nanoparticles has not new and it occurred throughout human history. However, it abruptly increased while the industrial and medical revolutions. The Nobel Prize Laureate Richard Zsigmondy first proposed the concept of “nanometer”. He created the term nanometer openly for characterizing particle size, moreover, he was the first to measure the size of particles such as gold colloids using a microscope [1].

Furthermore, modern nanotechnology was the brainchild of Richard Feynman, who was a Nobel Prize Laureate in 1965 for physics. American Physical Society meeting at Caltech was held in 1959 where he presented a lecture titled, “There’s Plenty of Room at the Bottom”. In that event, he explained and introduced the concept of manipulating matter at the atomic level. This novel idea demonstrated new ways of thinking and Feynman’s hypotheses have since been proven correct. Consequently, for this reason, he is considered the father of modern nanotechnology [2].

Approximately after15 years of Feynman’s lecture, a Japanese scientist, Norio Taniguchi, was the first to use “nanotechnology” to describe semiconductor processes that occurred in order of a nanometer. He explained that nanotechnology comprises the processing, separation, consolidation, and deformation of materials by one atom or one molecule. In the 1980s the most important and golden era of nanotechnology has begun when Kroto, Smalley, and Curl discovered fullerenes and Eric Drexler at the Massachusetts Institute of Technology (MIT) [3]. The Coming Era of Nanotechnology.” Drexler proposed the idea of a nanoscale “assembler” which would be able to build a copy of itself and other items of arbitrary complexity. Later on Drexler’s vision of nanotechnology is often called “molecular nanotechnology.” The science of nanotechnology was advanced further when Iijima, another Japanese scientist, developed carbon nanotubes [4].

In a limited timeframe of around half a century, nanotechnology has become the base for notable industrial applications and exponential growth. For instance, in the pharmaceutical industry, nanotechnology has had a thoughtful impact on medical devices such as diagnostic biosensors, drug delivery systems, and imaging probes. In the food and cosmetics industries, the use of nanomaterials has improved dramatically for developments in production, packaging, shelf life, and bioavailability. Zinc oxide quantum dot nanoparticles show antimicrobial activity against food-borne bacteria, moreover, nanoparticles are nowadays used as food sensors for detecting food quality and safety [5].

The safety of consumer products containing nanomaterials was an early general concern. It was expected that the risk assessment techniques used for drugs and toxic chemicals would be used for the risk evaluation of nanomaterials. However, reports of large data gaps indicated the need to augment conventional toxicity testing methods. Walker and Bucher summarized four reasons why nanomaterials need to be assessed differently than through the conventional methods [6]:

- New routes of exposure emerge when a nanomaterial is small enough to enter new cellular portals
- Surface properties impact dosimetry because they alter the toxico-kinetics of materials of similar size and shape
- The new commercial applications might lead to new biological interactions and unforeseen toxicities
- Assessment of relative risk using dose expressed in terms of mass may lead to false outcomes because some nanomaterials' dose can scale with a size-dependent property such as surface area.

The challenges are not only limited to the United States alone. Inappropriately, there is no internationally known standard protocol for toxicity testing of nanomaterials. Additionally, there are few if any internationally accepted well-characterized–positive controls available at present for nanomaterial studies [7].

Nanoparticles

Due to their very small size, nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plates, and sheets. This feature allows nanoparticles to possess unexpected optical, physical, and chemical properties, as they are small enough to restrict their electrons and produce quantum effects. Such as, copper is considered a soft material, with bulk copper bending when its atoms cluster at the 50*nm* scale. Therefore, copper nanoparticles smaller than 50*nm* are considered a very hard material, with extremely different malleability and ductility performance when compared to bulk copper [8]. Furthermore, change in size can also affect the melting characteristics; gold nanoparticles melt at much lower temperatures (300 °C for 2.5 *nm* size) than bulk gold (1064 °C). Moreover, absorption of solar radiation is much higher in materials composed of nanoparticles than in thin films of continuous sheets of material [9].

Classification of Nanoparticles

There are various approaches to the classification of nanomaterials. Nanoparticles are classified based on one, two, and three dimensions.

One-dimension nanoparticles: One-dimensional systems, such as thin film or manufactured surfaces, have been used for decades in electronics, chemistry, and engineering. Production of thin films (sizes 1-100 nm) or monolayer is now commonplace in the field of solar cells or catalysis. These thin films are used in different technological applications, including information storage systems, chemical, and biological sensors, fiber-optic systems, the magneto-optic and optical devices [10].

Two-dimension nanoparticles: Carbon nanotubes are a hexagonal network of carbon atoms, 1 *nm* in diameter and 100 *nm* in length, as a layer of graphite rolled up into a cylinder. CNTs are of two types, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The small dimensions of carbon nanotubes, combined with their remarkable physical, mechanical and electrical properties, make their unique materials. They display metallic or semi-conductive properties, depending on how the carbon leaf is

wound on itself. The current density that nanotubes can carry is extremely high and can reach one billion amperes per square meter making them a superconductor. The mechanical strength of carbon nanotubes is sixty times greater than the best steel. Carbon nanotubes have a great capacity for molecular absorption and offer a three-dimensional configuration. Moreover, they are chemically and chemically very stable [11].

Three-dimension nanoparticles: Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms, and contain C 60. This is a hollow ball composed of interconnected carbon pentagons and hexagons, resembling a soccer ball. Fullerenes are a class of materials displaying unique physical properties. They can be subjected to extreme pressure and regain their original shape when the pressure is released. These molecules do not combine, thus giving them the major potential for application as lubricants. They have interesting electrical properties and it has been suggested to use them in the electronic field, ranging from data storage to the production of solar cells. Fullerenes are offering potential applications in the rich area of nanoelectronics. Since fullerenes are empty structures with dimensions similar to several biologically active molecules, they can be filled with different substances and find potential medical applications [12].

Dendrimers: Another type of polymeric NP is dendrimers. Dendrimers are regularly branched macromolecules made from synthetic or natural elements including amino acids, sugars, and nucleotides. They have a central core, interior layers of branches, and an exterior surface.16 the varied combination of these components can yield dendrimers of well-defined size, shape, and branching length/density. As a result of their unique design, dendrimers can be developed as sensors as well as drug and gene delivery carriers. Dendrimers can be loaded with small molecules in the cavities of the cores through chemical linkage, hydrogen bond, and or hydrophobic interaction. The exterior surface can also be readily modified to produce chemical functional groups for molecular targeting groups, detecting and imaging agents, and therapeutic attachment sites [13].

Iron oxide: Iron oxide NPs are widely studied as passive and active targeting imaging agents as they are mainly superparamagnetic. The superparamagnetic iron oxide NP (SPION) generally has an iron oxide core with a hydrophilic coat of dextran or another biocompatible compound to increase its stability. The most widely used SPIONs consist of a magnetite (Fe_3O_4) and/or magnetite (γFe_2O_3) core. These NPs exhibit size-dependent superparamagnetic, which allows them to become magnetized with the application ofan external magnetic field and exhibit zero net magnetization upon removal of the magnetic field. SPIONs have been successfully used as T2 weighted magnetic resonance (MR) contrast agents to track and monitor cells. SPIONs have several advantages over conventional gadolinium-chelate contrast agents including decreased toxicity and increased imaging sensitivity and specificity [48]. SPIONS can also be degraded to iron and iron oxide molecules that are metabolized, stored in cells as ferritin, and incorporated into hemoglobin. Currently, two SPIO agents, peroxides (120–180 *nm*) and ferucarbotran (60 *nm*)are clinically approved for MRI. SPIONs have also been used in molecular imaging applications such as the detection of apoptosis and gene expression. SPIONs can be functionalized with magnetic, optical, radionuclide, and specifictargeting ligands for multimodal imaging. They can also potentially be used as non-invasive diagnostic tools and as drug delivery vehicles [14].

Quantum DOT: First discovered in 1980, quantum dots (QDs) are semiconductor particles that are less than 10 *nm* in diameter. QDs display unique size-dependent electronic and optical properties. Most QDs studied consist of a cadmium selenide (CdSe) core and a zinc selenide (ZnS) cap. The absorption spectra of these particles are very broad, and emission is confined to a narrow band. QDs can also emit bright colors, have long lifetimes, high efficiencies, and are stable against photobleaching. They can be generated to have different biochemical specificities and can be simultaneously excited and detected. As a result, QDs have several significant advantages over many organic fluorophore dyes for optical applications. They are widely used in biological research as fluorescence imaging tools for applications such as cell labeling and biomolecule tracking. The small size of quantum dots also

enables them to be suitable for biomedical applications such as medical imaging and diagnostics (15).

Characterization of Nanoparticles

Nanoparticles are generally characterized by their size, morphology, and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). The average particle diameter, size distribution, and charge affect the physical stability and the in vivo distribution of the nanoparticles. Electron microscopy techniques are very useful in ascertaining the overall shape of polymeric nanoparticles, which may determine their toxicity. The surface charge of the nanoparticles affects the physical stability and dispersibility of the polymer dispersion as well as their in vivo performance [16].

Particle size: Particle size distribution and morphology are the most important parameters for the characterization of nanoparticles. Morphology and size are measured by electron microscopy. The major application of nanoparticles is in drug release and drug targeting. It has been found that particle size affects drug release. Smaller particles offer a larger surface area [17].

Dynamic light scattering (DLS): Currently, the fastest and most popular method of determining particle size is photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS). DLS is widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. Shining monochromatic light (laser) onto a solution of spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light [18].

Scanning Electron microscopy: Scanning electron microscopy (SEM) is giving morphological examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis; however, they provide limited information about the size distribution and true population average [19].

Transmission electron microscope: TEM operates on a different principle than SEM, yet it often brings the same type of data. The sample preparation for TEM is complex and time-consuming because of its requirement to be ultra-thin for the electron transmittance [20].

Atomic force microscopy: atomic force microscopy (AFM) offers ultra-high resolution in particle size measurement and is based on a physical scanning of samples at the sub-micron level using a probe tip of atomic scale. The instrument provides a topographical map the of sample based on forces between the tip and the sample surface.

Surface Charge: The nature and intensity of the surface charge of nanoparticles are very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The colloidal stability is analyzed through the zeta potential of nanoparticles. This potential is an indirect measure of the surface charge. It corresponds to the potential difference between the outer Helmholtz plane and the surface of shear. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion [21]. High zeta potential values, either positive or negative, should be achieved to ensure stability and avoid aggregation of the particles. The extent of surface hydrophobicity can then be predicted from the values of zeta potential. The zeta potential can also provide information regarding the nature of material encapsulated within the nanocapsules or coated onto the surface.

Review of literature

The term nanotechnology was first defined by Tokyo Science University Professor Norio Taniguchi in 1974 as ‘’Nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule” [22]**.** The development of experimental techniques for the synthesis of NPs with regulated sizes, shapes, and properties is known as nanotechnology.

Materials differences at the nanoscale’s scales and their implications and potential benefits to human health. Nanotechnology is the science that deals with matter at the scale of 1 billionth of a meter (i.e., 10 − 9 m = 1 *nm*), and is also the study of manipulating matter at the atomic and molecular scale. It is an emerging field of science that includes the synthesis and development of various nanomaterials. Nanoparticles can be defined as objects ranging in size from 1- 100 nm that due to their size may differ from the bulk material. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate, and silver. Nanoparticles are being used for diverse purposes, from medical treatments, used in various branches of industrial production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes.

Nanotechnology (sometimes shortened to "nanotech") is the science that deals with the synthesis, characterization, exploration, and application of Nano-sized materials for the development of science. Nanotechnology, alongside nanostructured materials, plays an ever-increasing role in science, research, and economics everyday life, as more and more products based on nanostructured materials are introduced to the market [55,56]. It is a field that has arisen as a crossroads of biotechnology and nanotechnology for developing novel biosynthetic devices and environmentally friendly nanomaterial synthesis technology [23].

The study of phenomena and manipulation is known as nanoscience. The material at the atomic and molecular level characteristics differs dramatically from those at a bigger scales scale. It is a multidisciplinary science that spans several fields.

Nanotechnology, like semiconductor technology, information technology, and cellular and molecular, is expected to have a significant impact on our economy and society in the early twenty-first century. Nanotechnology is the creation and utilization of physical, chemical, and biological nanomaterials. tiny atom or molecules to large-scale chemical and biological systems nanostructures into bigger structures, as well as their integration into submicron dimensions' systems. Nanotechnology science and technology research promises advancements in areas such as materials and manufacturing, Nanoelectronics medicine and healthcare, energy, and biotechnology are just a few examples. National security and information technology nanotechnology is commonly believed to be the future.

The following Industrial Revolution A nanoparticle is defined as a collection of atoms linked together with a radius ranging from 1 to 100nm it usually has 10-105 atoms. The area of nanotechnology. Nanotechnology offers opportunities for the development of materials, particularly those for medical applications, where traditional methods, may have reached their limits. Drug delivery devices having a unique polymeric membrane are known as Nanocapsules. That is important in almost every field of science and technology. Nanotechnology has numerous scientific uses, and information technology as a result, we give a comprehensive review in this article a broad summary of NPs, their characteristics, and medicinal applications in several disciplines of science and technology, with a focus on their therapeutic possibilities [60, 61]. Nanotechnology has a wide range of scientific and technological applications. As a result, in this study, we provide a comprehensive overview of NPs in general, their properties, and therapeutic applications in various domains of science and technology, with a focus on therapeutic applications. It has already been applied in various fields, such as computer electronics, communication, energy production, medicines, and the food industry.

Nanotechnology can be applied by two different approaches, either “bottom-up” or top-down'. The top-down approach is achieved by employing physical processing of the food materials, such as grinding and milling.'' Bottom-up'' is the term used to describe starting with a single atom and molecules to build nanostructures. The concept describes technologies enabling the construction

of methods and tools to budding small components starting from larger regular matter.

Nanotechnology and Nanoscience got an improvement in the early 1980s with two main developments including both the birth of cluster science and the invention of the Scanning Tunneling Microscope (STM) in 1981. These developments caused the discovery of Fullerenes in 1985 and when Carbon Nano-tubes were advanced by Japanese scientists in 1991. In the medial of 1980s and beginning 1990s, several significant discoveries were made, which had an essential impact on the additional development of nanotechnology. For example, in 1991, the first Nano technological program of the National Scientific Fund was started to operate in the USA, and then in 2001, the National Nano technological Initiative (NNI) of the USA was approved. Since then, lots of technical research developments and scientific departments have taken place all over the world, particularly in some countries such as England, Japan, China, Germany, France, South Korea, and recently in the CIS countries [24].

History and Development of Nanotechnology

The term nanotechnology has been explained by a wide spectrum of numerous technologies that nanotechnology covers, which are based on many kinds of chemical, physical, and biological processes realized at the Nano-level [10]. The accurately established period for the beginning of nanotechnology development is demonstrated by the fact that nanotechnology has its background in the remote past when was used by people without knowledge of it.

The name “nanotechnology” was introduced by Norio Taniguchi for the first time in Tokyo in 1974 at the International Conference on Industrial Production to explain the super-thin processing of materials with nanometer accuracy and the establishment of Nano-sized mechanisms. Concepts of the Nanotechnological approach were put forward by Richard Feynman (identified as the Father of Nanotechnology) in 1959 in his lecture delivered at the session of the American Physical Society and were developed in 1986 by Eric Drexler.

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Consequently, the nanotechnology pattern was formed at the turn of the 1960s, whereas the 1980s and 1990s are the starts of the growth of nanotechnology in its own right. According to the light of the toxicity testing in the 21st century suggested by the US National Research Council (NRC), high-throughput screening of nanomaterials looks promising and may be potential in the not too distant future. Despite the complex nature of the nanomaterials which makes the development of their safety assessment challenging, the future of nanotechnology sounds to be bright [13-15].

Even though nanotechnology seems like a budding aspect of science, its utilization by humanity isn't novel at all. The history of nanomaterials usage in construction dates back to 4500 years ago when natural asbestos nanofibers were utilized for ceramic matrices. One of the oldest, richest, and most progressive cultures globally, Egyptians, realized the capabilities of nanomaterials 4000 years ago. The journey of nanomaterials and nanotechnology made throughout history before millennial technological advances used was given briefly in tabular form.

Nanoparticles

The prefix Nano is derived from the Greek word Nanos meaning "dwarf" or extremely small18. Nano-sized materials, known as NPs, possess unique and improved properties because of their larger surface area to volume ratio. NPs can be broadly grouped into two, namely, organic NPs and inorganic NPs which

include noble metal NPs (like silver and gold), and semi-conductor NPs (like titanium oxide and zinc oxide).

In general, nanoparticles used in the field of biotechnology range in particle size between 10 and 500 nm, seldom exceeding 700 nm. The nanosize of these particles allows various communications with biomolecules on the cell surfaces and within the cells in a way that can be decoded and designated to various biochemical and physicochemical properties of these cells. Similarly, its potential application in drug delivery systems and noninvasive imaging offered various advantages over conventional pharmaceutical agents. To utilize nanoparticles at their full throttle, the Nanoparticulate systems must be stable, biocompatible, and selectively directed to specific sites in the body after systemic administration. More specific targeting systems are designed to recognize the targeted cells such as cancer cells.

This can be achieved by conjugation of the nanoparticle with an appropriate ligand, which has a specific binding activity concerning the target cells. In addition, nanoparticles provide a platform to attach multiple copies of therapeutic substances to it and hence increase the concentration of therapeutic and diagnostic substances at the pathological site. Also, the concentration and dynamics of the active molecule can be varied by controlling the particle size of nanoparticles (>3–5 *nm*). This control in particle size in conjugation with surface coating with stealth ligand allows them to veil against the body's immune system, enabling them to circulate in the blood for a longer period.

These advances in the field of biotechnology have opened an endless opportunity for molecular diagnostics and therapy. Once targeted (active or passive), these nanocarriers can be designed in a way to facilitate them to act as imaging probes using a variety of techniques such as ultrasound (US), X-ray, computed tom(CT), positron emission tomography (PET), magnetic resonance imaging (MRI),), optical imaging, and surface-enhanced Raman imaging (SERS) [68].

Hence, these so-called "molecular imaging probes" can noninvasively provide valuable information about differentiate abnormalities in various body structures

and organs to determine the extent of the disease, and evaluate the effectiveness of treatment. Thus short molecular imaging enables the visualization of the cellular function and the follow-up of the molecular process in living organisms without perturbing them.

Over the year nanoparticles such as magnetic nanoparticles (iron oxide), gold and silver nanoparticles, nanoshells, and nanocages have been continuously used and modified to enable their use as diagnostic and therapeutic agents. Thus, in this particular review article, we have introduced iron oxide, gold, and silver nanoparticles along with newer nanoshells and nanocages. These are then briefly discussed for their method of development and some cite recent examples which utilize their intrinsic properties as diagnostic and/or therapeutic agents for diseases, mainly cancer [25].

Types of Nanoparticles

There are many types of NP platforms with differing sizes, shapes, compositions, and functionalities. Furthermore, each type of NPs can potentially be fabricated using different techniques, such as both nanoprecipitation and lithography for polymeric NPs.

Silver Nanoparticles: Silver nanoparticles have proved to be most effective because of their good antimicrobial efficacy against bacteria, viruses, and other eukaryotic micro-organisms. They are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions, etc. Studies have already reported the successful biosynthesis of silver nanoparticles by plants such as Azadirachta indica, capsicum annuum, and Carica papaya.

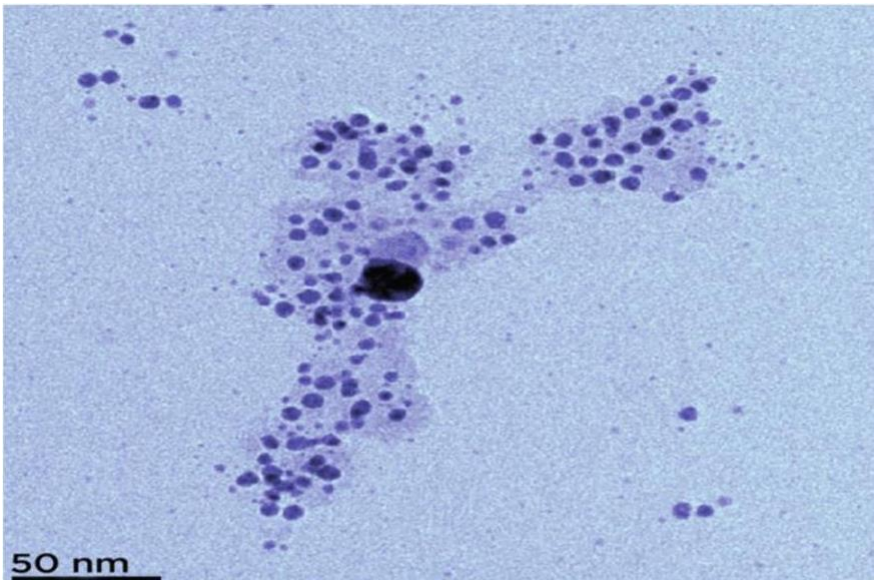

Fig. 1. Silver Nanoparticles

Gold Nanoparticles: Gold nanoparticles (AuNPs) are used in immunochemical studies for the identification of protein interactions. They are used as lab tracers in DNA fingerprinting to detect the presence of DNA in a sample. They are also used for the detection of aminoglycoside antibiotics like streptomycin, gentamycin, and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and identification of different classes of bacteria.

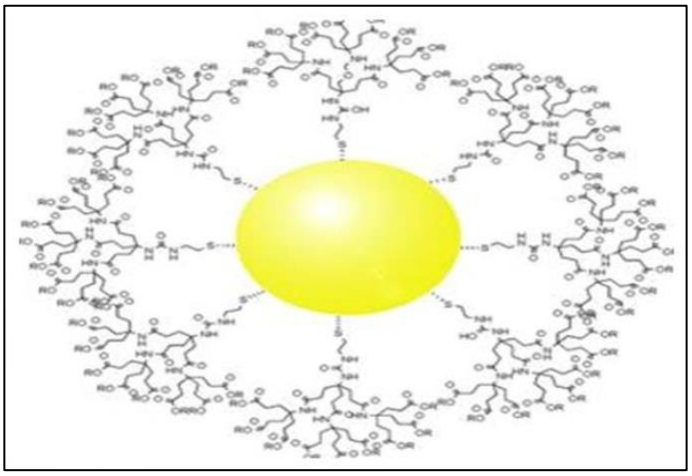

Fig. 2. Gold Nanoparticles

Alloy Nanoparticles: They exhibit structural properties that are different from their bulk samples. Since Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity, Ag flakes are most widely used. Bimetallic alloy nanoparticle's properties are influenced by both metals and show more advantages over ordinary metallic NPs.

Magnetic Nanoparticles: Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (MRI) [26].

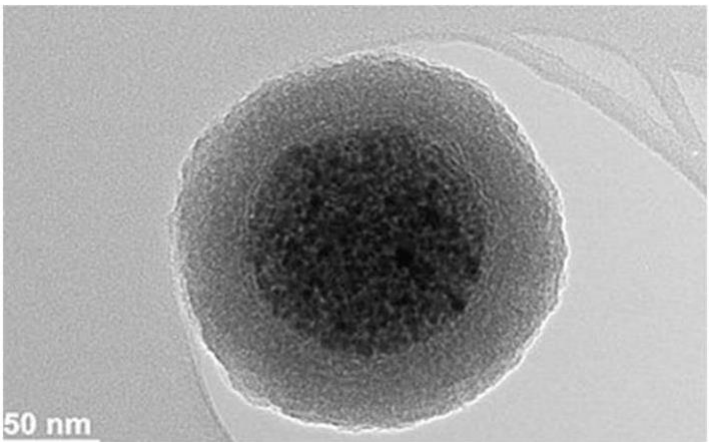

Fig. 3. Magnetic Nanoparticles

Liposomes Nanoparticles: The first NP platform was the liposomes. Liposomes were first described in 1965 as a model of cellular membranes. Since then, liposomes have moved from a model in biophysical research to one of the first NP platforms to be applied for gene and drug delivery. Liposomes are spherical vesicles that contain a single or multiple bilayer structure of lipids that self-assemble in aqueous systems. Unique advantages imparted by liposomes are their diverse range of compositions, ability to carry and protect many types of biomolecules, as well as their biocompatibility, and biodegradability. These advantages have led to the well-characterized and wide use of liposomes as transfection agents of genetic material into cells (lipofection) in biology research. Lipofection generally uses a cationic lipid to form an aggregate with the anionic genetic material.

Another major application of liposomes is their use as therapeutic carriers since their design can allow for the entrapment of hydrophilic compounds within the core and hydrophobic drugs in the lipid bilayer itself. To enhance their circulation half-life and stability *in vivo*, liposomes have been conjugated with biocompatible polymers such as polyethylene glycol (PEG). Liposomes can also be functionalized by targeting ligands to increase the accumulation of diagnostic and therapeutic agents within desired cells. Today, there are twelve clinically approved liposome-based.

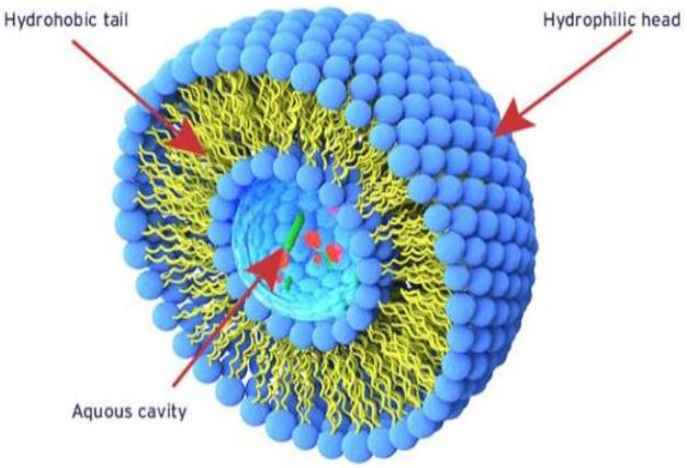

Fig. 4. Liposome Nanoparticles

Albumin-bound Nanoparticles: Albumin-bound NPs (nab) use the endogenous albumin pathways to carry hydrophobic molecules in the bloodstream. Albumin naturally binds to the hydrophobic molecules with non-covalent reversible binding, avoiding solvent-based toxicities for therapeutics. As a result, this platform has been successfully adapted as a drug delivery vehicle. Abraxane, 130 *nm* nab-paclitaxel was approved by the FDA in 2005 for the treatment of metastatic breast cancer. Abraxane concentrates in cells through albumin receptor (gp60) - mediated transport in endothelial cells. It may also target the albumin-binding protein SPARC (secreted protein acidic and rich

in cysteine), which is over-expressed in certain tumors. Further understanding of the mechanism of action may lead to better targeting and development of novel therapeutics using the nab platform.

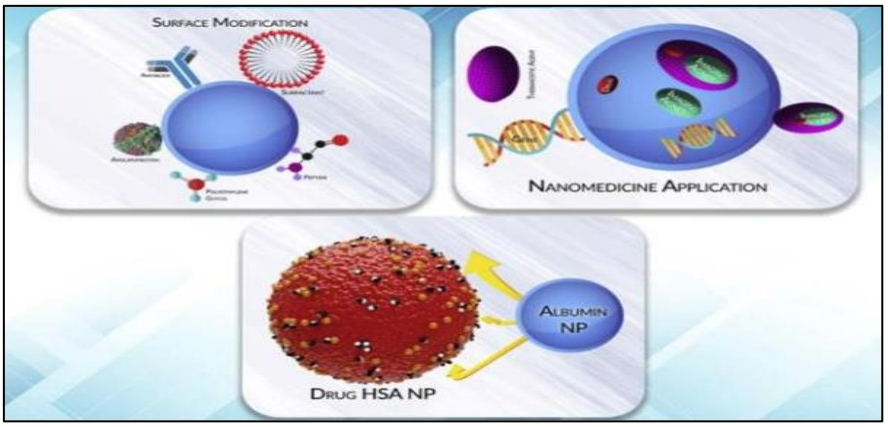

Fig. 5. Albumin Nanoparticles as nanocarriers for drug delivery.

Polymeric Nanoparticles: Polymeric NPs formed from biocompatible and biodegradable polymers have been extensively investigated as therapeutic carriers. Polymeric NPs are formulated through block-copolymers of different hydrophobicity. These copolymers spontaneously assemble into a core-shell micelle formation in an aqueous environment. Polymeric NPs have been formulated to encapsulate hydrophilic and/or hydrophobic small drug molecules, as well as proteins and nucleic acid macromolecules [26]. The NP design can allow for the slow and controlled release of drugs at target sites. Polymeric NPs are usually able to improve the safety and efficacy of the drugs they carry. Functionalizing polymeric NPs by targeting ligands for improved drug delivery has been important and can lead to their increased uptake along with their cargo, leading to enhanced therapeutic outcomes. Area of investigation since polymeric NPs are unique in their ability to be tailored before particle assembly. The incorporation of targeting ligands on the NPs.

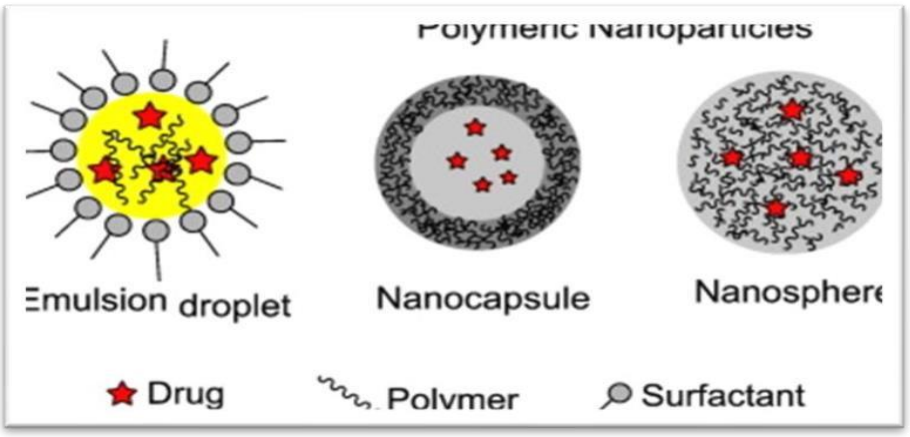

Fig. 6. Polymeric Nanoparticles

A Summary of classification of Nanoparticles

Table 1. Illustrated the various types of nanoparticles characterization and application;

Types of Nanosystems	**Size (nm)**	**Characteristics**	**Applications**
Carbon nanotubes	0.5–3 diameter and 20–1000 length	A third allotropic crystalline form of carbon sheets is either a single layer (single-walled nanotube, SWNT) or multiple layers (multiwall nanotube, MWNT). These crystals have remarkable strength and unique electrical properties (conducting, semiconducting, or insulating)	Functionalization enhanced solubility, and penetration to cell cytoplasm and nucleus, as a carrier for gene delivery, and peptide delivery.
Dendrimer	<10	Highly branched, nearly monodisperse polymer system produced by controlled polymerization; three	Long circulatory, controlled delivery of bioactive, targeted delivery of bioactive to macrophages, liver Targeting

		main parts core, branch, and surface	
Liposome	50–100	Phospholipid vesicles, are biocompatible, versatile, have good entrapment efficiency, and offer easy	Long circulatory, offer passive and active delivery of gene, protein, peptide, and various other
Metallic nanoparticles	<100	Gold and silver colloids, are very small in size resulting in a high surface area available for functionalization, Stable	Drug and gene delivery, highly sensitive diagnostic assays, thermal ablation, and radiotherapy enhancement
Nanocrystals Quantum dots	2–9.5	Semiconducting material synthesized with II-VI and III-V column elements; Size between 10 and 100 Å; Bright fluorescence, narrow emission, road UV excitation, and high photostability	The long-term multiple color imaging of liver cells; DNA hybridization, immunoassay; receptor-mediated endocytosis; labeling of breast cancer marker HeR 2 surface of cancer cells
Polymeric micelles	10–100 nm	Block amphiphilic copolymer micelles, high drug entrapment, payload, biostability	Long circulatory, target-specific active and passive drug delivery, the diagnostic value
Polymeric nanoparticles	10–1000	Biodegradable, biocompatible, offer complete drug protection	Excellent carrier for controlled and sustained delivery of drugs. Stealth and surface-modified nanoparticles can be used for active and passive

			delivery of bioactive

Synthesis of Nanoparticles

After several decades of intense research effort, it is apparent that a large number of synthesis approaches to a great variety of nanoparticles are available. Here we will exclusively focus on liquid-phase routes. In addition, due to space limitations, we restrict the number of examples to just a few that are representative of a specific class of materials and that are still frequently used. Nanofabrication methods can be divided roughly into two groups: top-down and bottom-up methods. Top-down methods start with patterns made on a large scale and reduce their lateral dimensions before forming nanostructures. On the other hand, bottom-up methods begin with atoms or molecules to build up nanostructures, in some cases through smart use of self-organization.

Top-down methods: In this method, a destructive approach is employed. Starting from the larger molecule, which decomposed into smaller units, and then these units are converted into suitable NPs. Examples of this method are grinding/milling, CVD, physical vapor deposition (PVD), and other decomposition techniques. This approach is used to synthesize coconut shell (CS) NPs. The milling method was employed for this purpose and the raw CS powders were finely milled for different intervals of times, with the help of ceramic balls and a well-known planetary mill. They showed the effect of milling time on the overall size of the NPs through different characterization techniques. It was determined that with the time increases the NP's crystallite size decreases, as calculated by the Scherer equation. They also realized that with each hour increment the brownish color faded away due to the size decrease of the NPs. The SEM results were also in an agreement with the X-ray pattern, which also indicated the particle size decreases with time.

One study revealed the spherical magnetite NPs synthesis from natural iron oxide (Fe_2O_3) ore by a top-down destructive approach with a particle size that varies from 20 to 50 nm in the presence of organic oleic acid. A simple top-down route was employed to synthesize colloidal carbon spherical particles with control size. The synthesis technique was based on the continuous chemical adsorption of polyoxometalates (POM) on the carbon interfacial surface. Adsorption made the carbon black aggregates into relatively smaller spherical particles, with high dispersion capacity and narrow size distribution. It also revealed from the micrographs, that the size of the carbon particles becomes smaller with sonication time. A series of transition-metal dichalcogenide nanodots (TMD- NDs) were synthesized by a combination of grinding and sonication top-down techniques from their bulk crystals. It was revealed that almost all the TMD-NDs with sizes.

In analogy with micromachining,26 top-down methods for nanomachining can be subdivided into 3 categories:

- Bulk/film-machining;
- Surface-machining;
- Mold-machining

- **Bulk/film machining**: In bulk-/film-machining the channel is created by etching trenches in the substrate wafer or the film deposited on the substrate. This is done typically by standard photolithography followed by wet or dry etching of the substrate in the case of substrate (bulk) etching and usually chemical etching of the film in the alternative approach, i.e., film etching.

  Surface-machining: In surface-machining first a bottom layer is deposited on the wafer followed by the deposition of the sacrificial layer and its patterning. Then, the top layer is deposited on top of the sacrificial layer and patterned (often with irrigation holes, which provide the access to the sacrificial layer). The nanochannel is finally formed by removing, i.e., etching the sacrificial layer leaving the bottom and the top layer to form the walls of the nanochannel. The bottom layer is not always required. It is mainly introduced to form the channel of one material (the same material as the top layer.

- **Mold-machining**: In principle, in mold-machining first the mold in the inverse shape of the desired structure is formed. This is filled with a structural material and then the mold can be etched or removed leaving the desired structure behind. The mold machining is mainly performed by soft lithography. In soft lithography, the mold is usually made by producing a pattern (master) in a layer of photoresist on the surface of the silicon wafer by photolithography or electron beam lithography (EBL). Then a liquid precursor to polydimethylsiloxane (PDMS) is poured over it and cured into the rubbery solid. The PDMS stamp is then peeled off the master. The fabrication of the master is rather expensive due to use of the electron-beam lithography or other advanced techniques.

  Copying the pattern on the PDMS stamp as well as the use of the stamp is, however, cheap and easy.

  Bottom-up methods: In such methods, the atoms and molecules are assembled into the smallest nanostructures (dimensions of typically 2 to 10 nm) by carefully controlled chemical reactions, which make this technique cheaper as compared to the lithographical methods.

  This approach is employed in reverse as NPs are formed from relatively simpler substances, therefore this approach is also called the building up approach.

  Examples of this case are sedimentation and reduction techniques. It includes sol-gel, green synthesis, spinning, and biochemical synthesis. Mogilevsky et al. synthesized TiO_2 anatase NPs with graphene domains through this technique. They used alizarin and titanium isopropoxide precursors to synthesize the photoactive composite for photocatalytic degradation of methylene blue. Alizarin was selected as it offers strong binding capacity with TiO_2 through their axial hydroxyl terminal groups. The anatase form was confirmed by the XRD pattern. SEM indicates that with temperature elevation, the size of NPs also increases (Mogilevsky et al., 2014). Well-uniform spherical-shaped Au nanospheres with monocrystalline have been synthesized via laser irradiation top-down technique (fig No. 8).

Liu et al. selectively transform the octahedra morphology to a spherical shape by controlling the laser treatment time and other reaction parameters. Fig No.8 provides the SEM and TEM of the prepared Au nanospheres, which showed an average diameter of 2.6 *nm* of Au nanospheres in edge length of Au octahedra per particle, more recently, the solvent-exchange method is used to achieve limit-sized low-density lipoprotein (LDL) NPs for medical cancer drug delivery purpose by Needham et al. In this method nucleation is the bottom approach followed by growth which is the up approach. The LDL NPs were obtained without using phospholipid and possessed high hydrophobicity, which is essential for drug delivery applications. The monodispersed spherical bismuth (Bi) NPs were synthesized by both top-down and bottom-up approaches. These NPs have excellent colloidal properties. In the bottom-up approach, bismuth acetate was boiled within ethylene glycol, while in the top-down approach the bismuth was converted into molten form, and then the molten drop was emulsified within the boiled diethylene glycol to produce the NPs. The size of the NPs obtained by both methods varied from 100 nm to 500 *nm*. Green and biogenic bottom-up synthesis attracts many researchers due to the feasibility and less toxic nature of the processes. These processes are cost-effective and environmental friendly, where synthesis of NPs is accomplished via biological systems such as using plant extracts. Bacteria, yeast, fungi, Aloe vera, tamarind, and even human cells are used for the synthesis of NPs. Au NPs have been synthesized from the biomass of wheat and oat and using the microorganism and plant extracts as reducing agents.

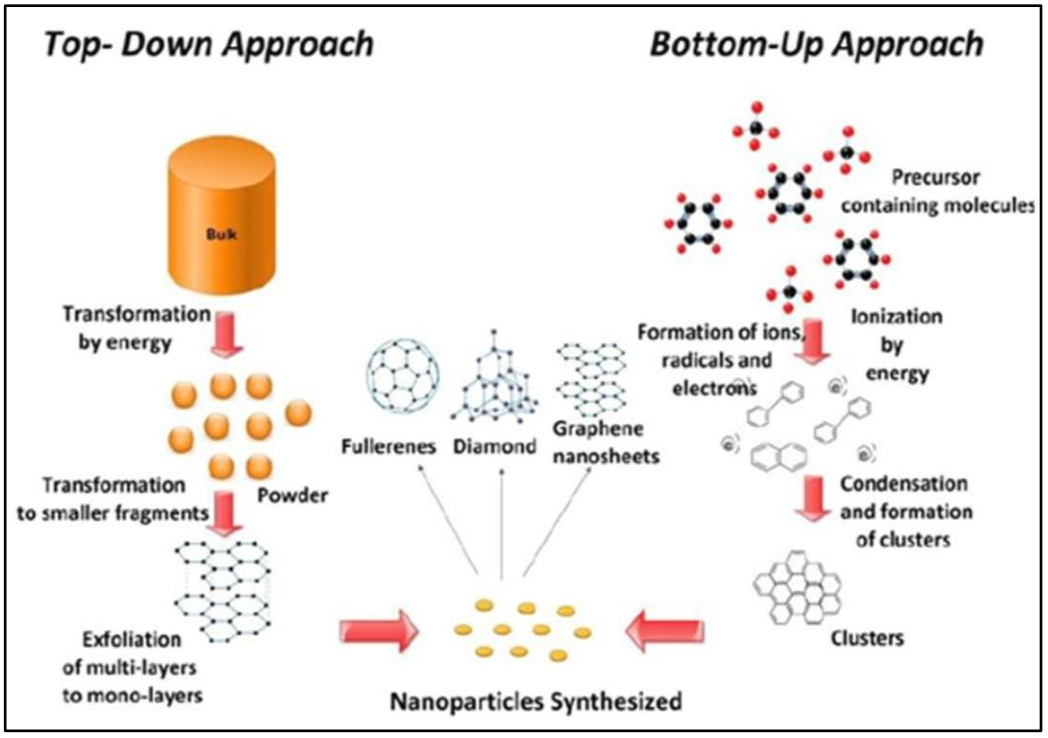

Fig. 7. Depicts the nanoparticles synthesis by Top-Down & Bottom-up approaches.

Overview of synthesis of Nanoparticles

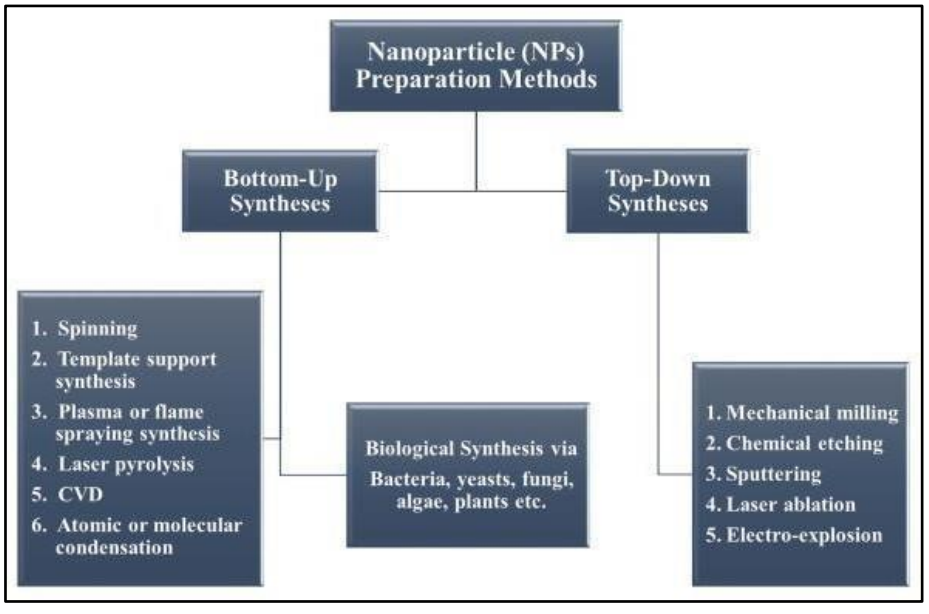

Fig. 8. Typical synthetic methods for NPs for the top-down and bottom-up approaches

Gold Nanoparticles Synthesis

When it comes to the synthesis of gold nanoparticles, probably the most convenient and widely used synthesis technique is the so-called citrate route developed more than 60 years ago. The reaction is very simple and involves just gold chloride, sodium citrate as a reducing and stabilizing agent, and water as a solvent. The obtained gold nanoparticles exhibit a spherical morphology with a relatively narrow size distribution (20 ± 1.5 *nm*). Although the citrate route is still very popular for the preparation of aqueous gold nanoparticle sols, many other approaches have been developed, performed in specific organic solvents or the presence of different types of surfactants and reducing agents to fine-tune the morphology from nanocubes) to hexagonal shapes nanorods) and nanostars. Interestingly, all these nanoparticles have different optical properties. In the case of gold nanorods with varying aspect ratios, this effect is visible to the naked eyes. The optical absorption spectra show a shift of the band at longer wavelengths, corresponding to the absorption and scattering of light along the long axis of the nanorods, from the visible to near-infrared with increasing lengths, and the color of the corresponding dispersions change from pink to blue, green, and brownish.

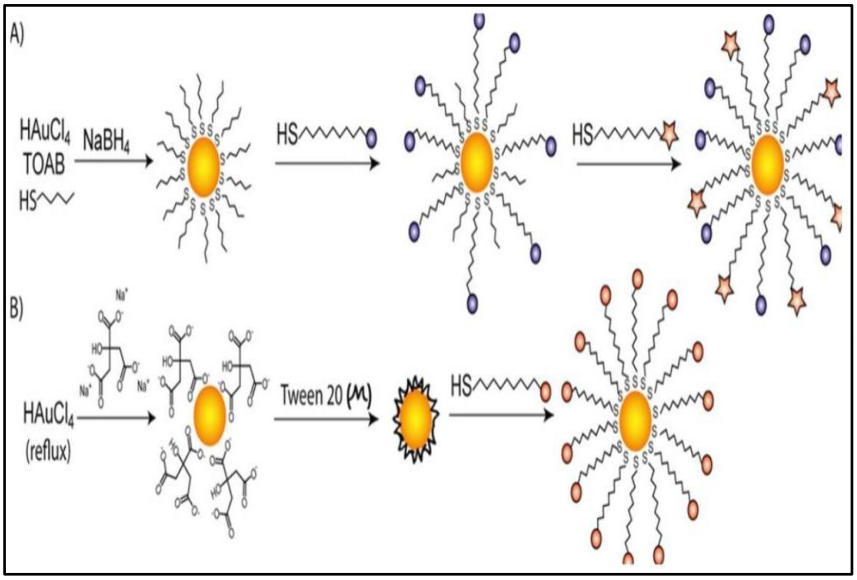

Fig. 9. (A) Two-phase synthesis of AuNPs by reduction of HAuCl4 in presence of alkanethiols as the stabilizing ligands and NaBH4 as the reducing agent. Place-exchange reaction for alkanethiol-protected AuNPs can then be performed with functionalized thiols. **(B)** Citrate-stabilized AuNPs were prepared with $HAuCl_4$ solution under reflux conditions where citrate acts as both the stabilizing ligand and reducing agent. The ligand exchange of functionalized thiols for citrate-stabilized AuNPs was achieved by using Tween 20 as an intermediate.

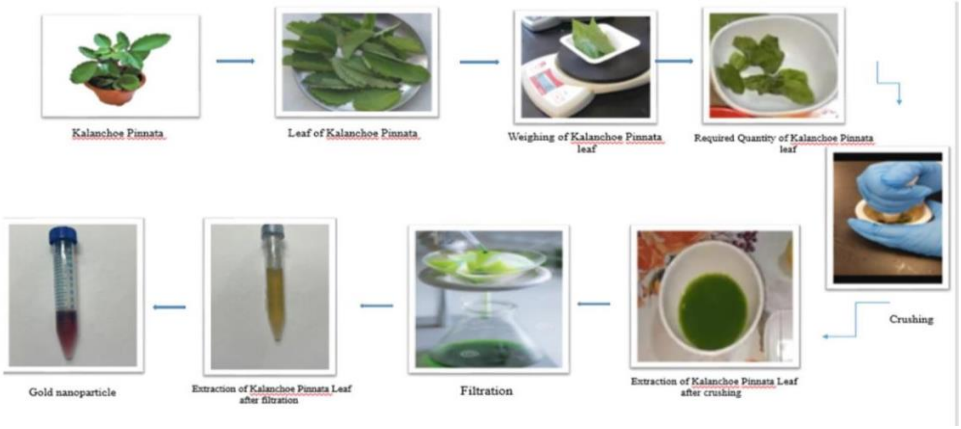

Fig. 10. An overview of the synthesis of gold Nano-particle from Kalanchoe Pinnata leaves.

Properties and application of gold Nanoparticles

Spherical AuNPs possess useful attributes such as size- and shape-related optoelectronic properties, large surface-to-volume ratio, excellent biocompatibility, and low toxicity. These properties make AuNPs an

important tool in bionanotechnology. Important physical properties of AuNPs include surface plasmon resonance (SPR) and the ability to quench fluorescence. Spherical AuNPs exhibit a range of colors (e.g., brown, orange, red, and purple) in aqueous solution as the core size increases from 1 to 100 nm, and generally show a size-relative absorption peak from 500 to 550 nm. This absorption band arises from the collective oscillation of the conduction electrons due to the resonant excitation by the incident photons which is called a “surface plasmon the band” [27].

Properties	**Application Area**
Redox activity	Electronic devices and electrochemical sensing
Surface-enhanced Ramanscattering (SERS)	Imaging and sensing
Surface Plasmon resonance (SPR)	Sensor fabrication and materials science

Sensing: AuNPs are readily conjugated with recognition moieties such as antibodies or oligonucleotides for the detection of target biomolecules, allowing *in vitro* detection and diagnostics applications for diseases such as cancer. As an example, AuNPs play a critical role in the “bio-barcode assay”, an ultrasensitive method for detecting target proteins and nucleic acids. The principle of the “bio-barcode assay” utilizes AuNPs conjugated with both barcode oligonucleotides and target-specific antibodies, and magnetic microparticles (MMPs) functionalized with monoclonal antibodies for the target moiety.

These complexes produce a sandwich complex upon detection of the target molecule that releases a large number of barcode oligonucleotides, providing both identification and quantification of the target.

Therapeutics: The transport of therapeutic agents to the cells by AuNPs is a critical process in biomedical treatment. Several research groups have used functionalized AuNPs to investigate the interactions with the cell membrane to improve delivery efficiency.

Imaging: The versatile optical and electronic properties of AuNPs have been employed for cell imaging using various techniques, including computed tomography (CT), dark-field light scattering, optical coherence tomography (OCT), photothermal heterodyne imaging technique, and Raman spectroscopy.

Application & future perspective of nanoparticles

Due to the capability to produce the materials in a particular way to play a precise role, the use of nanomaterials spans a wide variety of industries, from healthcare and cosmetics to environmental preservation and air purification. The healthcare field, for example, uses nanomaterials in myriad ways, such as drug delivery [19,20]. Whereby nanoparticles are being developed to assist the transportation of chemotherapy drugs directly to cancerous growths, as well as to deliver drugs to areas of arteries that are damaged to fight cardiovascular disease, and carbon nanotubes are used in processes such as the addition of antibodies to the nanotubes to create bacteria sensors. Further use of nanomaterials in this industry can be identified in the use of antimicrobial nanotechnology in items such as the towels and mats used by sportspeople, to prevent illnesses caused by bacteria. Apart from the healthcare sector in aerospace, carbon nanotubes can be used in the morphing of aircraft wings. Additionally, zinc oxide nanowires play a role in the treatment of polluted water.

Elsewhere in the cosmetics industry titanium oxide nanomaterial is used in sunscreen, due to the poor stability that conventional chemical UV protection offers in the long term [21,22].

Nanomaterials have also been developed for use in the military. One example is the use of mobile pigment nanoparticles being used to produce a better form of camouflage, through injection of the particles into the

material of soldiers' uniforms. Additionally, the military has developed sensor systems using nanomaterials, such as titanium dioxide, that can detect biological agents [23, 24].

Nanoparticles have already been applied as drug delivery systems with great success. Nanoparticles provide massive advantages regarding drug targeting and delivery and with their potential for combined diagnosis and therapy and are one of the major tools in nanomedicine. There are many technical, challenges in developing the following techniques [25].

- Virus-like systems for intracellular systems
- Architecting of biomimetic polymers
- control of sensitive drugs, functions (of active drug targeting, bioresponsive triggered systems, systems interacting with my body smart delivery)
- nanochips for nanoparticle release
- carriers for advanced polymers for the delivery of therapeutic peptides/proteins.
- Drug delivery techniques were established to deliver or control the amount & rate.

Kalanchoe Pinnata

Kalanchoe is a genus that has many species most of which are used as agents to treat various ailments. Plants belonging to this genus have been traditionally known for their pharmaceutical value and have been studied by scientists for a very long time. Kalanchoe pinnata (synonym: Bryophyllum pinnatum) commonly known as "Ranakalli" "Miracle leaf", "Mexican Love plant", "Katakataka", "Cathedral Bells", "Air plant", "Life plant", "Goethe plant", "Wonder of the World" and so on belongs to the Crassulaceae family. It is also known as the "Mother of thousand" as new plantlets arise from the leaf. margins which can be cut off from the parent and cultivated separately on pots or barren lands [26].

Fig. 11. Kalanchaoe Pinnata Plant.

Scientific Classification

Kingdom:	*Plantae*
Family:	*Crassulaceae*
Genus:	Kalanchoe
Species:	K. *pinnata*

This plant is a water-storing perennial that grows about 1 to 1.5 m tall. The leaves are thick green, fleshy, and distinctively scalloped. The stems are tall and hollow-bearing pendulous bell-like flowers. This plant is mostly found in plains, tropical and temperate regions of Africa, Australia, and America. It is one of the ethnomedical used medicinal plants in the folklore of Asia.

Earlier in Africa, K. pinnata was used to facilitate childbirth, and treat ulcers, skin diseases, and rheumatism.

The parts of the plant are supposed to be closely connected with characteristics of ‘astrality’ corresponding to the soul organization in humans, controlling the hysteria (excess of activity) of the metabolic-limb system. In vitro, experimental studies showed antihistaminic activity of this plant. Those studies also demonstrated improvement in sleep quality in pregnant women, and tocolytic effects similar to the beta-agonist but with fewer adverse effects.

Benefits of *Kalanchoe pinnata*

Kalanchoe and its benefits are well documented throughout the gardening world. It is a beautiful plant with huge leaves, so it is known as the “tree of happiness “This plant has many medicinal uses including treating insomnia, arthritis, anxiety, and depression. It also strengthens the immune system, detoxifies the body, and improves mental health.

Pharmacological effects of the plant

Wound-healing activity: The ethanolic extract of *K. pinnata* showed significant wound-healing activity by decreasing the size of the affected area as well as edema at the wounded site. This may be due to the presence of steroidal glycosides and phenolic antioxidants. A study carried out proved that water, petroleum ether, and alcoholic extracts of the plant have the potential to heal wounds. The study also demonstrated that water extract showed more activity than the other two extracts.

Antioxidant activity: Antioxidative agents protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. Antioxidants possess reducing properties generally associated with the presence of reductones, which have shown to exert antioxidant action by breaking the free radical chain either by donating a hydrogen atom or an electron. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Potential antioxidant activity has good correlations with the treatment of cardiovascular disorders.

The leaves were reported to show maximum scavenging effects than stems and the ethanolic extract showed more total phenolic and flavonoid content than other extracts. The high amount of phenols and flavonoids in the extracts may be the reason for their high antioxidative activity. The phenolic constituents can interact with the transition metals even in the lipid phase and chelate them by filling their aqua-coordination sites and generating metal-coordinated insoluble complexes. Inhibition of lipid auto- oxidation could be attributed to the ability of phenolic to stabilize the radicals through the generation of stabilized phenoxyl radicals by directly scavenging peroxyl radicals. *K. pinnata* has a strong protective potential than standard antioxidants against oxidative stress in both aqueous and lipid phases. The metal-chelating ability (aqueous phase) of the extract was found to be dependent on its ability to reduce metal-induced peroxidative stress (lipid phase) [27].

Anti-tumor activity: Methanol and aqueous extracts of the plant were administered as drugs in rats with specific dosages. These extracts decreased the ascetic fluid volume and arrested the tumor growth, acting as a tumor-suppressing agent. Thus, the extracts were reported to possess antitumor activity. The extract exhibited apoptosis-inducing properties, and growth-inhibitory activity, on cervical cancer cells due to the presence of certain phyto compounds. The protective effect may be due to the antioxidant and antiperoxidative effects coupled with an ability to correct the abnormalities in lipid and lipoprotein metabolism through an increase in the activities of a few lipids metabolizing enzymes. N-diethylnitrosamine (DENA) tends to generate free radicals as its metabolism takes place in the liver, disturbing the antioxidant status, ultimately leading to oxidative stress and carcinogenesis, and that is the reason it is considered a major environmental hepatic carcinogen. Histopathological examination of the liver section of DENA-treated rats showed intense centrilobular necrosis and vacuolization. The aqueous extract scavenged the free radicals, reduced necrotic damage, and protected the hepatocytes from the carcinogenic effects of DENA [28].

Antiviral activity: Human papillomavirus (HPV) is one of the sexually transmitted viruses, acting as a major threat to humans. Cervical cancer which is on the rise is caused by HPV. A study by Mahata et al., (2012) examined the anticancer and anti-HPV activities of chloroform extract from the plant. The extract fractions when subjected to cancer cell lines, suppressed the expression of viral proteins thus inhibiting viral as well as tumor growth. The Epstein-Barr virus is a herpes virus that affects the B-lymphocytes of humans, leading to the formation of tumors.

Antimicrobial activity: Two flavonoids and an alkaloid from the ethanolic leaf extract of *K. pinnata* proved the antimicrobial activity of the plant. These phytocompounds inhibited the growth of some commonly found gram-negative and positive bacteria and fungi. Ogochukwu (2011) demonstrated the antimicrobial ability of aqueous and methanolic extracts of the plant stem. Pattewar et al., (2013) studied the antimicrobial potential of *K. pinnata* and stated that methanolic extract showed a better inhibition rate. Bacteria that are found on the skin can cause skin infections and when they enter the body, they cause respiratory diseases, food poisoning, wound infections, abscesses, osteomyelitis, endocarditis, pneumonia, and other complications. So, the prepared extract can act against such diseases, and save the lives of infected ones. The reported data can be used to prepare antibacterial and antifungal creams for commercial use (Pattewar et al., 2013). Mudi and Ibrahim (2008) isolated three bioactive compounds from leaf extract and tested their activity against respiratory infection-causing pathogenic bacteria.

The research confirmed the traditional use of the plant for curing respiratory tract infections including pneumonia. Chowdhury et al., (2011) worked on petroleum ether and aqueous extracts of K. pinnata to study the antifungal and cytotoxic activities of the extracts. It was reported that both the extracts showed the almost same effect as that of the antifungal agent used as a standard [29].

Antileishmanial activity: The protozoans of the genus Leishmania

cause the disease Leishmaniasis. The aqueous extract of *K. pinnata* was given orally to mice infected with Leishmania amazonensis. After the trial, few observations were made such as the decrease in size of the lesions and the parasitical load in the infected area.

Continuous treatment with the extract not only controlled the growth but also prevented the further occurrence of the infections. It was suggested that this method could be applied to visceral leishmaniasis also (Muzitano et al., 2009). The antileishmanial activity may be attributed to the presence of flavonoid glycosides in the extract of the plant (Muzitano et al., 2006).

Anti-allergic activity: Cruz et al., (2012) studied the effect of *K. pinnata* on mast cells. Mast cells play a pivotal role in the development of allergic asthma. The study showed that the aqueous extract of the plant effectively inhibited mast cell degranulation, thus preventing the development of allergic airway diseases. The data reported suggest that the extract can also act as an immunosuppressive agent. Allergic anaphylaxis is another life-threatening immune reaction that causes death under extreme situations. Continuous administration of aqueous extract of *K. pinnata* prevented acute events related to allergen-induced anaphylaxis.

Muscle-relaxant activity: Salahdeen and Yemitan (2006) tried evaluating the muscle-relaxant activity of the aqueous extract and observed a reduction in the muscle tone of the laboratory animals. The spasmolytic activity was studied by Ozolua et al., (2010) who presented the antispasmodic effect of aqueous leaf extract on tracheal smooth muscle cells and that the extract could be used for the prophylaxis of asthma. Nwose (2013) determined the effect of the ethanolic extract on serum creatine kinase. An increase in the values of creatine kinase activity in the albino rats treated with the ethanolic extract of the plant was observed. This increase in activity could encourage the supply of energy (ATP) needed for muscular contraction and relaxation, which in the case of asthma can bring about dilatation of constructed smooth muscles of the bronchi.

Antipyretic activity: Biswas and Montal (2015) demonstrated the effect of plant extract on hyperthermic conditions in laboratory animals. Pyrexia was induced in rats by injecting Brewer's yeast. When hydroalcoholic extract of *K. pinnata* was administered to the laboratory specimens, it reduced the body temperature thus exhibiting its antipyretic effect. The presence of flavonoids in the extract may be the reason for this activity.

Antilithiatic activity: The reduced oxalate excretion in the urine causes the formation of calcium oxalate stones. Fresh juice extracted from the leaves of *K. pinnata* was administered to patients having stones in their bodies based on medical prophylaxis. Regular intake of the juice effectively dissolved the stones regardless of their position, nature, and previous treatments. There was an increase in the quantity of urine excreted, thus showing the diuretic nature of the juice. It also facilitated the decrease in oxalate excretion, while increasing citrate excretion. This study suggests that the juice may have antilithiatic properties (Gahlaut et al., 2012). Shukla et al., (2014) evaluated the anti-urolithiasis effect of aqueous extract on ethylene glycol-induced renal calculi in rats. Histopathological examination of the kidneys showed reduced renal damage, less degeneration of epithelial lining, and tubular dilatation in all the extract-treated rats. Gilhotra et al., (2013) formulated tablets of *K. pinnata* extracts and reported that the drug is effective in controlling the accumulation of calcium oxalate crystals and preventing stone formation in the kidneys.

Antidiabetic activity: Diabetes is a major risk for cardiovascular diseases such as stroke, and heart attack which affects the majority of the global population. Goyal et al., (2013) reported that the ethanolic extract of *K. pinnata* decreased the blood glucose level of rats affected by diabetes. Thus, decreasing the serum glucose level and increasing glucose tolerance. The plant extract also increased the pancreatic secretion of insulin. Matthew et al., (2013c) investigated the antidiabetic activity of ethanolic and aqueous extracts of the dried stem of K. pinnata against alloxan-induced diabetes in rats.

They reported that both the extracts exhibited hypoglycemic activity, that is, significant antihyperglycemic activity [30]. The activities of both the extracts were compared and it was observed that the ethanolic extract showed more inhibitory activity of α-amylase enzyme than the aqueous extract, while the aqueous extract showed more hypoglycemic activity than the ethanolic extract. Ojewole (2005) determined the hypoglycaemic activity of aqueous extract of the plant by inducing diabetes in rats through streptozotocin treatment. Once the aqueous extract was orally administered, a reduction in blood glucose level was observed. And, after 24- hour continuous observation, the glucose levels subsided to normal baseline levels indicating the antidiabetic potential of the plant.

Objectives

Objective

- Green synthesis of AuNPs by using Kalanchoe pinnata aqueous leaf extract.
- Characterization of synthesized AuNPs by UV-vis spectroscopy, DLS, Zeta potential, and TEM.
- Antibacterial activity of synthesized AuNPs against Gram-positive & Negative Bacteria.

Materials and method

Materials

Tetra chloroauric acid ($HAuCl_4$) was purchased from Sigma Aldrich. Phosphate buffer salts (Na_2HPO_4) and (NaH_2PO_4) were purchased from HIMEDIA. Double distilled water has been used as an aqueous medium for all experiments. All buffers were filtered with 0.2µm filter paper immediately after they were prepared. Microbiological media and ingredients were purchased from Himedia, India. All solvents and chemicals were of analytical grade and used as obtained from Merck and Sigma–Aldrich (St. Louis, MO, USA)

Method

Preparation of leaf extract

Fresh *Kalanchoe pinnata* was collected from integral university Kursi Road (Lucknow, India) and used for the preparation of aq. extract. The leaf was peeled off from the plant and was cleaned with running tap water, freeze-dried and the bark was crushed in phosphate buffer (pH 7.2) using a pestle and mortar. The resultant extract was centrifuged at 6000 rpm for 10 minutes and then filtered by using Whatman filter paper no.1.

In vitro synthesis of AuNPs

In vitro synthesis of AuNPs was done by taking a reaction mixture of 3ml containing 30µl (diluted) of 1mM HAuCl4 salt in PBS buffer (pH was 7.2 and it was filtered by 0.2µm filter) and 0.48ml of freshly prepared *Kalanchoe pinnata* leaf aqueous extract. This extract was used as a source for the synthesis of AuNPs and served as a reducing agent and also provide stability to particles. The extract reduces Au (III) to Au (II) anions which were further reduced to form monodispersed, spherical Gold Nanoparticles of different sizes. On completion of the reaction, the synthesized Gold Nanoparticles were centrifuged for 5 minutes at 5000rpm. The supernatant and the pellet were separated with the help of a 0.2µm filter. This was followed by the characterization of AuNPs using the technique UV-vis Spectroscopy.

Antibacterial activity of synthesized Gold Nanoparticles

Preparation of growth media

For the preparation of media, 13.3 gm of MHA was taken in 350 ml of distilled

water in a conical flask and was sterilized for 15-20 minutes in the autoclave.

Preparation of bacterial culture plates-

The media was poured into the 4 culture plates to prepare the cultures of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Bacillus subtilus* and were kept at room temperature for solidifying (all the steps were taken out in aseptic conditions i.e., Laminar Air Flow)

- The 4 wells were created into the plates to pour the antibiotic sample (50µl) and Fresh plant extract (80µl) in 2 wells separately, Synthesized Gold Nanoparticles (80µl) in 3rd well and 4th well was left controlled.
- The antibiotic and Fresh Plant Extract was poured to check their efficacy when compared to Synthesized Gold Nanoparticles. (All the steps were carried out in Laminar Air Flow)
- The plates were kept in the incubator for 24 hours at 37°C

Results and discussion

Result

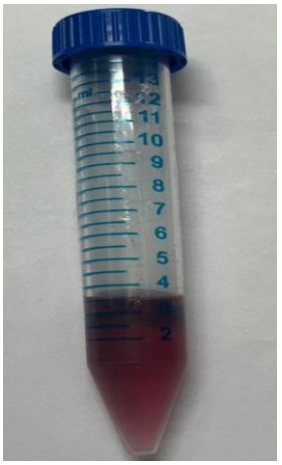

Fig. 12. Synthesized KP-AuNPs

Characterization of Gold Nanoparticles

Characterization is done by using UV- visible spectroscopy.

Characterization of KP-AuNPs

In noble metal nanoparticles, an unfamiliar phenomenon is observed due to surface plasmon resonance (SPR). This imparts the quality of intense electromagnetic fields onto the nanoparticle's surface, resulting in scattering and absorption. Thus, the formation of KP-GNPs was confirmed herein using U.V. vis spectra (Figure14). The absorption peak was observed at 528 nm, which corresponds to the SPR band of the KP-GNPs. The phytoconstituents in *Kalanchoe pinnata* leaf extract reduced the gold salt ($HAuCl_4$) into AuNPs and encapsulated the AuNPs, preventing the nanoparticles from aggregating and providing stability to the KP-AuNPs. The change in color from light yellow to ruby red indicated the successful synthesis of R-AuNPs, and the result of the SPR band confirmed that at 528 nm.

However, there was no discernible peak for *Kalanchoe pinnata* .leaf extract. The transmission electron microscope (TEM) was used to determine the precise size, shape, and 2-dimensional morphology of KP-AuNPs, which was determined to be 17 d. nm with spherical shape and monodispersed parameter (Figure. 15). The technique of dynamic light scattering (DLS) was used to determine the average particle size and profile of the particle size distribution of KP-AuNPs. KP-AuNPs had an average particle size of 68 d.nm and a polydispersity index (PDI) of 0.215, indicating a homogeneous size distribution. The zeta potential of KP-AuNPs was also investigated (Figure. 16). Generally, a zeta value of -

20 mV is needed for the colloidal stability of nanoparticles. The zeta potential of the prepared KP-AuNPs was -17 mV, indicating the high strength of the particles. When the aqueous dispersion of KP-AuNPs was observed at room temperature, no clumping or accumulation was observed. This was most likely due to the electrostatic repulsive forces of the gold nanoparticles. This repulsion prevents the nanoparticles from coming into contact with one another.

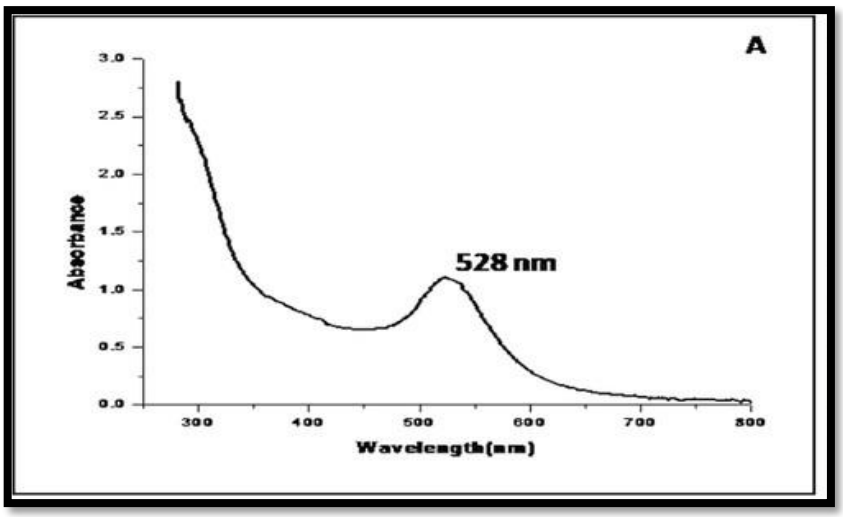

Fig. 13. UV-Vis Spectra of synthesized KP-AuNPs shows a peak at 528nm.

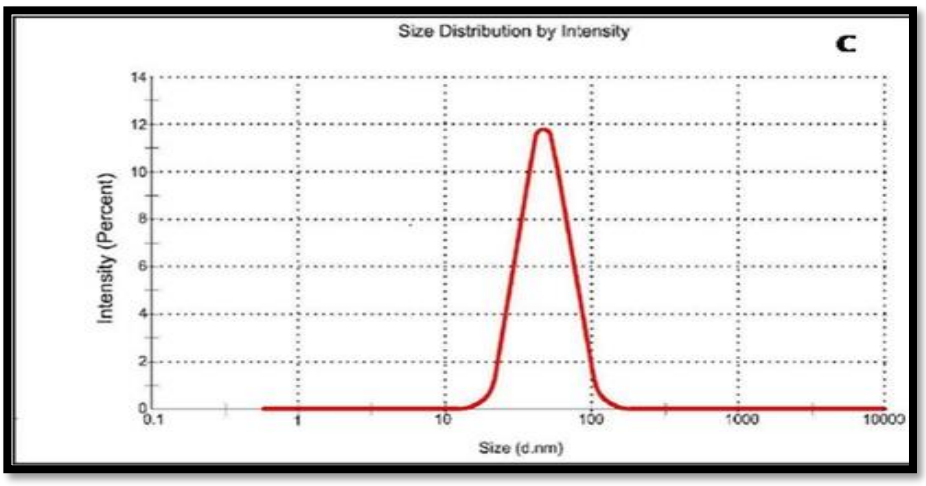

Fig. 15. Size distribution of colloidal KP Au-NPs at 68d. nm.

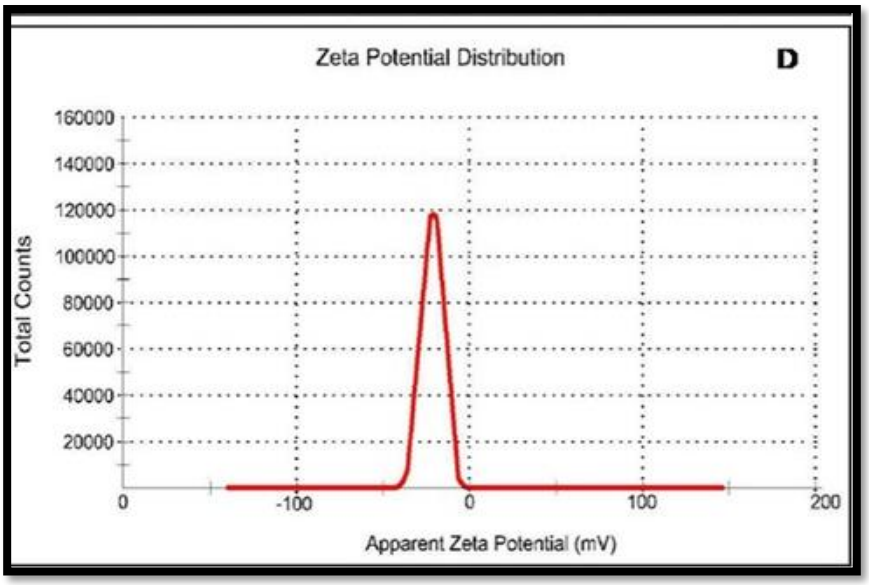

Fig. 16. The zeta potential shows at -17mV.

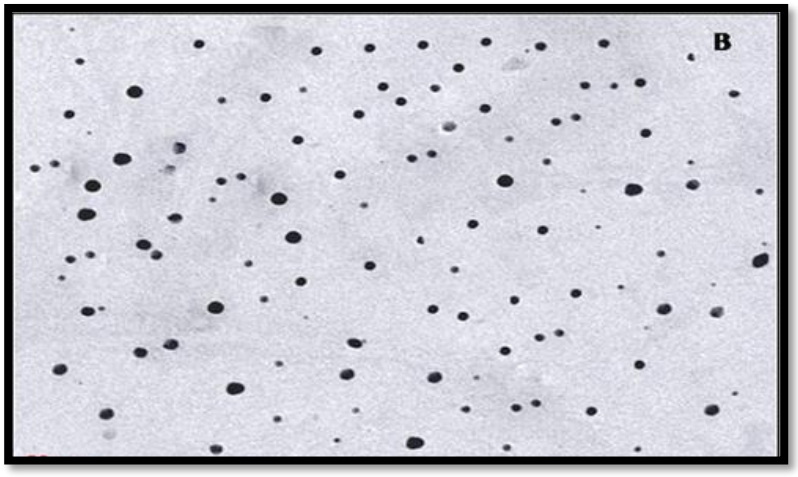

Fig.14. Transmission Electron Microscope illustrations of the precise size, shape, and 2-dimensional morphology of KP-AuNPs 17 d. nm.

Antibacterial activity

The synthesized KP-AuNPs was showing activity against *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis*, and *Streptococcus pyogenes* causing the zone of inhibition. The antibacterial activities of synthesized gold nanoparticles from *Kalanchoe pinnata* leaf extract against selected bacteria showed in figure 18.

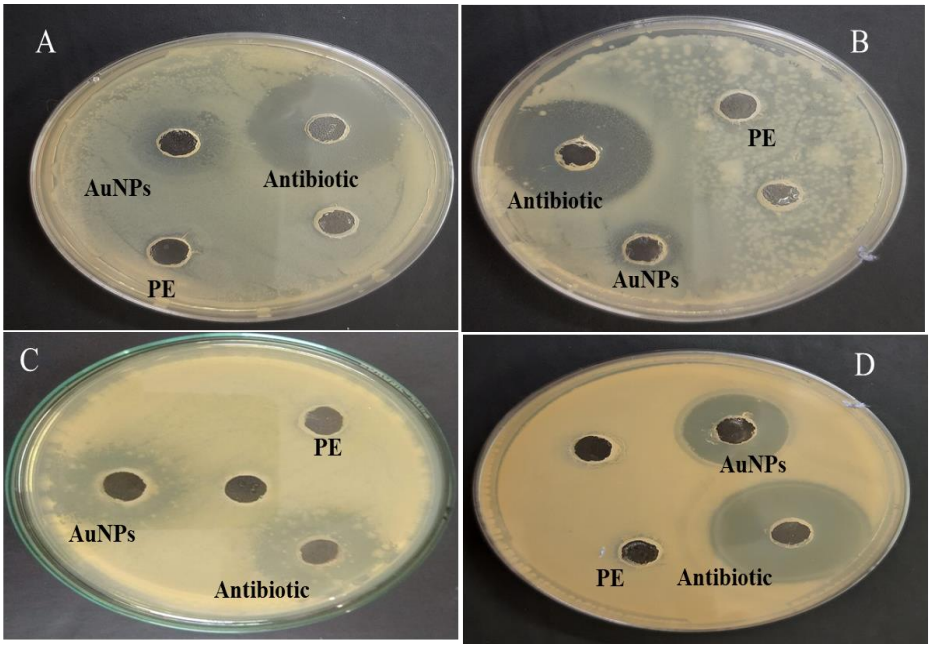

Fig. 17. Antibacterial activity against (A) Staphylococcus aureus (B) Escherichia coli (C) Bacillus subtilis, and (D) Streptococcus pyogenes respectively (Well diffusion method).

Determination of Minimal Inhibitory Concentration of *Kalanchoe pinnata* leaf extract and KP-AuNPs

The MIC is the lowest concentration of *Kalanchoe pinnata* leaf extract and KP-AuNPs that completely inhibit bacterial growth, and MIC_{50} is the concentration of *Kalanchoe pinnata* leaf extract and KP-AuNPs that inhibit 50% of the bacterial population. The MIC_{50} of *Kalanchoe pinnata* leaf extract and KP-AuNPs against several Gram-negative and Gram-positive bacterial strains were recorded. However, Amoxicillin was used as a standard antibiotic during the experiment. The quantified MIC_{50} values were: *Staphylococcus aureus*, 0.91 mg/ml, 0.61 mg/ml, and 1.15 mg/ml; *Escherichia coli*, 0.79 mg/ml, 0.36 mg/ml, and 1.02 mg/ml; *Bacillus subtilis*, 0.42 mg/ml, 0.27 mg/ml, and 0.474 mg/ml; *Streptococcus pyogenes*,

7.67 mg/ml, 3.86 mg/ml, and 8.5 mg/mL of pure *Kalanchoe pinnata* leaf extract, KP-AuNPs, and Amoxicillin (control), respectively. The findings, as mentioned earlier, indicated that KP-AuNPs had a 1.4-fold, 2.1-fold, 1.5-fold, and 1.9-fold increased antibacterial potential compared to pure *Kalanchoe pinnata* leaf extract when tested against *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis*, and *Streptococcus pyogenes,* respectively. However,

compared to the control (Amoxicillin), KP-AuNPs showed an enhanced antibacterial potential of 1.8 folds, 2.8 folds, 1.7 folds, and 2.2 folds against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus pyogenes*, respectively. The potent antibacterial activity observed might be due to the presence of phytoconstituents present in the plant. Recent studies reported that plant rich in alkaloids resembles a significant amount of antibacterial activity. Additionally, our extracts also contain flavonoids, tannins, saponins, gums, and mucilages in a significant amount which might be the reason for its extraordinary antibacterial potential.

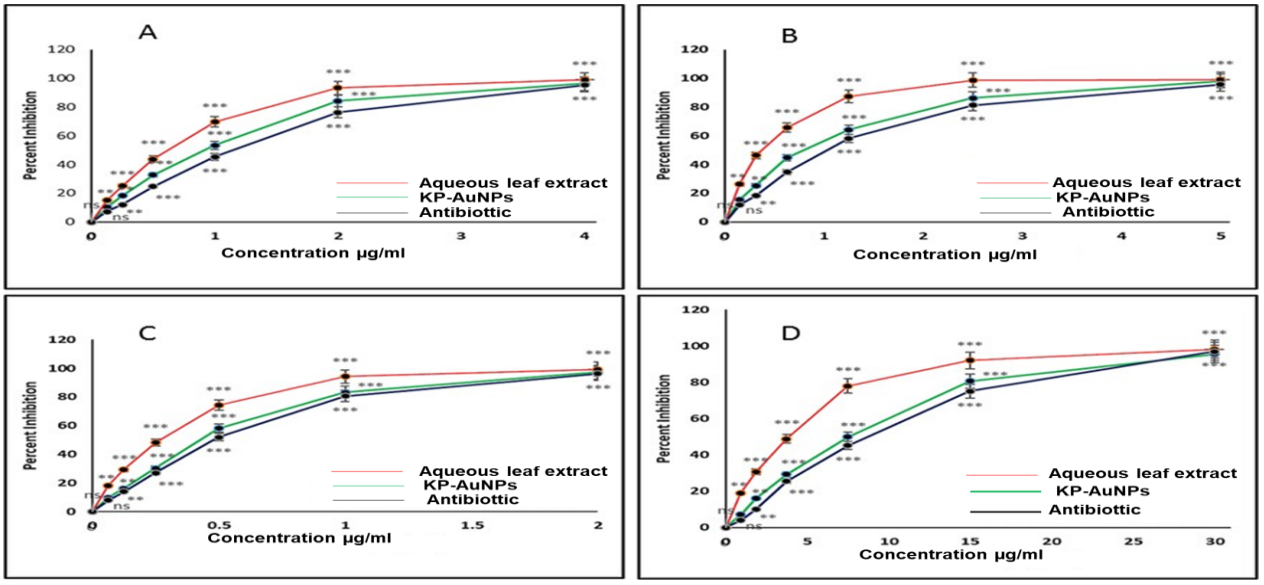

Fig. 18. Determination of Minimum Inhibitory Concentration (MIC) of pure Kalanchoe pinnata aqueous leaf extract, KP-AuNPs, and Amoxicillin (positive control) against (A) Staphylococcus aureus (B) Escherichia coli (C) Bacillus subtilis, and (D) Streptococcus pyogenes.

Discussion and conclusion

Discussion

Kalanchoe pinnata was utilized as a reducing and stabilizing agent in this work. The synthesis of KP-AuNPs is thought to be triggered by *Kalanchoe pinnata* aqueous leaf extract's reducing enzymes and capping agents, such as secondary metabolites, which work together to decrease Au ions. The color of the synthesized AuNPs was Ruby Red color which is the characteristic feature of AuNPs. According to Mie Theory, Gold shows resonance known as Plasmons in the UV-visible spectrum. These resonances are formed by the interaction of electromagnetic waves and electrons at the surface of AuNPs. This resonance characteristic of AuNPs can be observed by spectroscopy. In this study, *Kalanchoe pinnata leaf* aqueous extract was used for the synthesis of KP-AuNPs. This extract was used as a source for the synthesis of KP-AuNPs and served as a reducing agent and also provide stability to the particles. This plant extract reduced Au (III) to Au (II) anions which were further reduced to form monodispersed, spherical Gold NPs of different sizes.

The characterization of synthesized KP-AuNPs was done by UV-visible spectroscopy and peak were found at 529nm, due to the surface plasmon resonance property of Gold Nanoparticles.

Therapeutic Analysis was performed for the KP-AuNPs for antibacterial assay, and multiple drug resistance for different species and found to be positive in most cases. A synthesized gold nanoparticle from *Kalanchoe pinnata leaf* aqueous extract shows broad inhibition against specific bacteria thereby making the synthesized gold nanoparticles a good antimicrobial agent.

Conclusion

Nanoparticle-based technologies cover different fields, ranging from environmental remediation, energy generation, development of potential drug molecules, etc. Nanoparticle characterization is necessary to establish an understanding and control of nanoparticle synthesis and applications. In this study, Gold Nanoparticles have been synthesized using *Kalanchoe pinnata* leaf extract. As a previous study, the plant has anticancer, antioxidant, antidiabetic, antibacterial, anti-Alzheimer, and nephroprotective activity.

Studies are underway to investigate the potential of GNPs in Diabetic complications as well as human studies and the work requires in-depth study to establish the Nanoparticle as a potent drug molecule.

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