A DISSERTATION ON

Efficacy of *Thuja Orientalis* plant-mediated synthesis of gold nanoparticles against different pathogens

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BY

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Adeeba Sheeri**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled ***"Efficacy of Thuja Orientalis plant-mediated synthesis of gold nanoparticles against different pathogens"*** 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.

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IU/DBS/S00192/2022/CIN/221108 **June 2022**

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Mr. Adeeba Sheeri**, 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 ***"Efficacy of Thuja Orientalis plant-mediated synthesis of gold nanoparticles against different pathogens"*** 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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Adeeba Sheeri **Date**

LIST OF ABBREVIATIONS

AuNPs	gold nanoparticles
Levo	levofloxacin
PBS	Phosphate buffer
M	Molarity
mM	Milli Molar
DLS	Dynamic Light Scattering
OD	Optical density
M deg	Milli degree
SPR	Surface Plasma Resonance
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
UV-Vis	Ultraviolet-Visible Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
ZP	Zeta potential
nm	Nanometer

Introduction

Introduction

Nanotechnology is the science, engineering, and technology at the nanoscale s about 1-100 *nm*. Nanotechnology manipulates and controls substances at the nano-level (1*nm* = one billionth of a meter) and creates new substances and devices by using the best properties of the nanoparticles. The concept behind nanoparticles started with a talk by physicist Richard Feynman on December 29, 1959, at an American Physical Society meeting at the California Institute of Technology (Caltech), Feynman described a process in which scientists in the future would be able to control and manipulate individual atoms and molecules. Professor Mario Taniguchi a decade later coined the term 'nanotechnology' through the exploration of ultra-precision machining.

Nanotechnology encompasses the application of physical, chemical, and biological systems at scales ranging from individual atoms and molecules and also the integration of these nanoparticles into larger systems. Nanotechnology has emerged as a multidisciplinary field in which understanding the electrical, optical, magnetic, and mechanical properties of nanostructures promises to deliver wide-ranging functional material applications. Nanostructures have also proved to provide solutions to technological and environmental challenges in the area of catalysis, medicine, solar energy conversion, and water treatment.

The development in metrology has also contributed to the rapid development of nanotechnology in the 1980's IAM group invented the scanning tunneling microscope that enables researchers to observe and verify various nanostructures.

Nanotechnology also has useful applications in the industrial field, in the IT sector scientists, are applying nanotechnology to the development of high-density memories/computer devices with new operating principles. Nanoparticle advances in the IT sector are usually in material solid things like; nanoparticles embedded steel by Arcelor Mittal. Nanotechnology is also been used to create advanced microchips. Nantero developed an NRAM microchip to replace the high-density flash microchip.

Nanotechnology is used in the medicinal field for high throughput drugdelivery systems, target drug delivery is another amazing application of nanotech particles are engineered such that they get conjugated with diseased cells only thereby treating diseased cells only, reducing damage tohealthy cells and helping in the early detection of diseases. Researchers at the University of Worcester are using antibodies attached to carbon nanotubes in chips to detect cancer cells in bloodstreams this method can beused in labs for the early of detection cancer cells in bloodstreams. Gold nanorods are being used in combination with infrared rays to sterilize hospitalinstruments in the future.

Nanotechnology also has the potential to save raw materials, energy, water, and greenhouse gas. Nanoparticles' distinctive properties can be used in various procedures that can assist environmental and climate protection. A photolytic copper tungsten oxide nanoparticle wh activated with sunlight breaks oil spills in oceans into biodegradable compounds saving the ocean and marine life from catastrophic damages.

Nanotechnology is a dynamic field that combines basic scientific inquiry and industrial application. Carbon nanotubes are 100% carbon and are compatible with cells and organic matter, they have electrical conductivity, thermal conductivity, and mechanical strength

There are two general strategies for the synthesis of nanomaterials: the top-down approach, wherein a larger structure is broken down into smaller pieces using chemical, physical, and biological energy; and the bottom-up approach, in which material is synthesized from the atomic level using various chemical, physical, or biological reactions to make a large nanostructure. The use of toxic chemicals for the synthesis of nanoparticles can cause hazardous effects like carcinogenicity, and environmental toxicity. The use of toxic solvents and chemical contaminations limits the use of nanoparticles in various clinical and biomedical applications. Hence a need for a cleaner, better, and environmentally friendly approach to the synthesis of nanoparticles is a necessity, the biological synthesis of nanoparticles includes multicellular and unicellular biological entities like bacteria, actinomycetes, fungi, plants, viruses, and yeasts.

Plants are the most suitable entities for the green synthesis of nanoparticles as they are non-pathogenic and various pathways of plants are researched. A wide range of metal nanoparticles has been produced using different plants. These nanoparticles have various optical, chemical, physical, thermal, and electrical properties as compared to their bulk parts and thus have various applications in nano forms. Various metal nanoparticles like Cu, Ag, Au, etc are synthesized using green synthesis. [de Marco, B.et al.2019]

NPs are a wide range of materials with dimensions below 100 nm, which can be used in various applications, such as medical, pharmaceutical, manufacturing and materials, environmental, electronics, energy collection, and mechanical industries, due to their multiple properties

In 2009, Raveendran et al. published the first green synthesis methods of metal NPs. With an aqueous starch solution subjected to heating, silver nitrate ($AgNO_3$), and glucose as the green reducing agent [After that, researchers like Iravani and Kumar et al. presented high-quality review papers regarding the synthesis of metallic NPs using plant extracts as a green chemistry approach

Unusual physical, chemical, and biological approaches for the synthesis and manufacturing of metal NPs have been created as a result of the nanotechnological boom. The novelty of this study lies in reporting the applicable green synthesis of AuNPs and AgNPs from plant extracts, as well as their capacity as antimicrobial agents in the agricultural field for combating bacterial and fungal pathogens that can cause plant, waterborne, and foodborne infections. Furthermore, this study presents a summary of the contributions of AuNPs and AgNPs to water treatment and the development of "environmentally friendly" nano fertilizers, nano pesticides, and nano herbicides, well as the negative implications of NP accumulation in plants and soils.

Numerous methodologies are developed to synthesize noble metal nanoparticles of particular shape and size depending on specific requirements. Biosynthesis of nanoparticles has an emerging highlight of the intersection of nanotechnology and biotechnology which has received increased attention to a growing need to develop environmentally benign technologies in material syntheses. Biomolecules as reductants are found to have a significant advantage over chemical reductants due to their non-biocompatible nature. [Huang J et al. 2007]

Green chemistry's versatility enables the creation of a diverse spectrum of organic and inorganic nanomaterials with several promising applications. In all circumstances, it is critical to thoroughly characterize the resulting nanomaterials, as their properties will determine their ability to execute the purpose for which they were synthesized, as well as any negative impacts.

Review of literature

Nanoparticle

Nanoparticles are microscopic objects with at least one dimension less than 100 *nm*. Due to their relatively large surface area, nanoparticles often exist with distinctive size-dependent properties Moreover, a particle at the nanoscale has a length smaller than de Broglie wavelength of the charge carrier (electrons and holes) or the wavelength of light. The physical properties of nanoparticles become quite different from bulk materials, which yields interesting and new applications. [Akbari B, et al.2011]

Platforms of nanoparticles

There are several different varieties of NP platforms, each with its size, shape, composition, and functionality. The following platforms for nanoparticles are discussed:

Liposomes: Liposomes are the first nanoparticle platform. Liposomes were first described as a model of biological membranes in 1965. Liposomes were then employed to carry genetic and pharmacological information. Liposomes can be utilized to target ligands to increase the accumulation of diagnostic and therapeutic substances within cells. There are now 12 clinically approved liposome-based medicinal medicines. [Bangham A. Liposomes et al.1993]

Albumin-bound: Albumin-Bound Nanoparticles (NAB) use the endogenous albumin trails which transport hydrophobic molecules in the bloodstream It quandaries with hydrophobic molecules with non-covalent reversible binding and dodging solvent-based toxicities for therapeutics. So, this platform has been adapted for drug delivery [Hawkins MJ, et al. 2008]

Polymeric: Polymeric nanoparticles are formed from biocompatible and biodegradable polymers which are used as therapeutic carriers. Polymeric nanoparticles are verbalized through block-copolymers of diverse hydrophobicity. These nanoparticle designs are useful because of the slow and controlled release of drugs at required sites. [Gref R, et al.1994]

Quantum dots: Quantum dots (QDs) are semiconductor particles and their size is less than 10 nm in diameter. QDs show unique size-dependent electronic and optical properties. Mostly the quantum dots consist of cadmium selenide (CdSe) as the core and zinc selenide (ZnS) as a cap (or shell). They are used in biological research as fluorescence imaging cell labeling and biomolecule tracking [Collier C, et al.1998].

Iron oxide: Iron oxide NPs are studied as passive and active targeting imaging agents because they are superparamagnetic. They have an iron oxide core with a hydrophilic coat of dextran or another biocompatible compound to increase their stability. They are mostly used in MRI. Till now, two SPIO agents, ferumoxides (120-180 nm) and ferucarbotran (60 nm) are clinically approved for MRI.

Classification of nanoparticles

There are various approaches to the classification of nanomaterials. Nanoparticles are classified based on one, two, and three dimensions. NMs can be created with various modulations dimensionalities: Pokropivyn and Skorokhod classified the nanostructured materials based on their dimensionalities as (1) zero-dimensional nanoparticles (2) - dimensional nanoparticles (3) two-dimensional nanoparticles (4) three-dimensional nanoparticles.

Zero-dimensional nanoparticles

A major feature that discriminates various types of nanostructures is their dimensionality. In the past 10 years, significant progress has been made in the field of 0 D NSMs. A rich variety of physical and chemical methods has been developed for fabricating 0D NMSs with well-controlled dimensions. Recently 0 D NMSs such as uniform particle arrays (quantum dots), heterogeneous particle arrays, core-shell quantum dots onions, hollow spheres, and Nano lenses have been synthesized by several research groups.

One-dimensional nanoparticles

In the last decade, 1D NSMs have stimulated an increasing interest due to their importance in research and development and have a wide range of potential applications. It is generally accepted that 1D NSMs are ideal

systems for exploring a large number of novel phenomena at the nanoscale and investigating the size and dimensionality dependence of functional properties. They are also expected to play an important role as both interconnects and the key units in fabricating electronics, optoelectronic, and EEDs with nanoscale dimensions. 1D NSMs have a profound impact on nanoelectronics, nanodevices and systems, nanocomposite materials, alternative energy resources, and national security.

Two-dimensional nanoparticles

2D nanostructures have two dimensions outside the nanometric size range. In recent years, the synthesis of 2D NSMs has become a focal area in materials research, owing to their many low dimensional characteristics different from the bulk properties. In the quest for 2D NSMs, considerable research attention has been focused over the past few years on the development of 2D NMSs. 2D NMSs with certain geometries exhibit unique shape-dependent characteristics and subsequent utilization as building blocks for the key components of nanodevices. In addition, 2D NSMs are particularly interesting not only for basic understanding of the mechanisms of nanostructures growth but also for investigating and developing novel applications in sensors, photocatalysts, nanocontainers, nanoreactors, and templates for 2D structures of other materials.

Three-dimensional nanoparticles

It is well known that the behaviors of NSMs strongly depend on the sizes, shapes, dimensionalities, and morphologies which are thus the key factors to their ultimate performance and applications. Therefore it is of great interest to synthesize 3D nanostructures with a controlled structure and morphology. In addition, 3D nanostructures are an important material due to their wide range of applications in the area of catalysis, magnetic materials, and electrode material for batteries. Moreover, the 3D NSMs have recently attracted intensive research interest because the nanostructures have a higher surface area and supply enough absorption sites for all involved molecules in a small space.

Characterization of nanoparticles

SEM, transmission electron microscopy, and other advanced microscopic techniques are used to characterize nanoparticles for their size, shape, and surface charge. Atomic force microscopy (AFM) and electron microscopy (TEM) (AFM).The average particle diameter, distribution of particle sizes, and charge impact the in vivo distribution and physical stability of the nanoparticles. Techniques of electron microscopy are extremely important in determining the form of polymeric nanoparticles, in general, Their toxicity may be determined. The charge on the surface of the. The physical stability and dispersibility of nanoparticles are affected. The dispersion of polymers as well as their in vivo performance.

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. As a result, most of the drug loaded onto them will be exposed to the particle surface leading to fast drug release. On the contrary, drugs slowly diffuse inside larger particles. As a drawback, smaller particles tend to aggregate during the storage and transportation of nanoparticle dispersion. Hence, there is a compromise between small size and the maximum stability of nanoparticles (Redhead et al., 2001).

Polymer degradation can also be affected by particle size. For instance, the degradation rate of poly (lactic-co-glycolic acid) was found to increase with increasing particle size in vitro (Betancor et al., 2000).

There are several tools for determining nanoparticle size as discussed below;

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 cause a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. It is possible to extract the size distribution and give a description of the particle's motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function. The photon correlation spectroscopy (PCS) represents the most frequently used technique for accurate estimation of the particle size and size distribution based on DLS (DeAssis et al., 2008).

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. For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons (Jores et al., 2004).

The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The nanoparticles must be able to withstand vacuum, and the electron beam can damage the polymer. The mean size obtained by SEM is comparable with results obtained by dynamic light scattering. Moreover, these techniques are time-consuming, costly, and frequently need complementary information about sizing distribution (Molpeceres et al., 2000)

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 electron transmittance. The nanoparticle dispersion is deposited onto support grids or films. To make nanoparticles withstand the instrument vacuum and facilitate handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, uranyl acetate, etc, or by plastic embedding. An alternate method is to expose the sample to liquid nitrogen temperatures after embedding it in vitreous ice. The surface characteristics of the sample are obtained when a beam of electrons is transmitted through an ultra-thin sample, interacting with the sample as it passes through (Molpeceres et al., 2000)

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 (Muhlen et al., 1996). The instrument provides a topographical map of the sample based on forces between the tip and the sample surface. Samples are usually scanned in contact or non-contact mode depending on their properties. In contact mode, the topographical map is generated by tapping the probe onto the surface across the sample and the probe hovers over the conducting surface in non-contact mode. The prime advantage of AFM is its ability to image non-conducting samples without any specific treatment, thus allowing imaging of delicate biological and polymeric nano and microstructures (Shi & Farber, 2003). AFM provides the most accurate description of size and size distribution and requires no mathematical treatment. Moreover, particle size obtained by the AFM technique provides a real picture which helps understand the effect of various biological conditions (Polakovic et al., 1999)

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. 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 Surface hydrophobicity: Surface hydrophobicity can be determined by several techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements, etc.

Recently, several sophisticated analytical techniques are reported in the literature for surface analysis of nanoparticles. X-ray photon correlation spectroscopy permits the identification of specific chemical groups on the surface of nanoparticles.

Drug Release: A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important. To obtain such information most release methods require that the drug and its delivery vehicle be separated. The drug loading of the nanoparticles is generally defined as the amount of drug bound per mass of the polymer (usual moles of drug per mg polymer or mg drug per mg polymer); it could also be given as a percentage relative to the polymer. The technique used for this analysis is classical analytical methods like UV spectroscopy or high-performance liquid chromatography (HPLC) after ultracentrifugation, ultra-filtration, gel filtration, or centrifugal ultrafiltration. Quantification is performed with UV spectroscopy or HPLC. Drug release assays are also similar to drug loading assay which is assessed for some time to analyze the mechanism of drug release (Magenhein et al., 1993).

Gold nanoparticle

GNPs are the most compatible nanomaterial for the preparation of engineered nanoplatforms in smart sensing devices. The surface Plasmon resonance property of GNP makes them the most suitable engineered nanomaterial for bioimaging, biomedical therapeutics, and bio diagnostics tools. [Jain, P.K., Lee, K.S., El-Sayed, I.H. and EL-Sayed, et al. (2006)]

GNP also named gold colloids, has attracted increasing attention due to their unique properties in multi-disciplinary research fields. [Daniel, M.C. and Astruc, D et al. (2004)]

Although GNPs are defined by tiny size, significant quantities of GNPs are likely required in many commercial and industrial applications.

Novel emerging applications bring huge growth to the global demand for GNPs. A biomolecule or biopolymer-conjugated GNPs are largely used as biomarkers and bio delivery vehicles in medicine/pharmacy and cosmetic products. GNPs are employed as antiaging components for skin protection. [Boisselier, E. and Astruc, D et al. (2009)]

GNPs are used to treat wool or cotton fibers for a permanent coloration of value textiles

GNPs are used to enhance the performance of non-volatile memory devices and low-temperature printing metal inks in electronics.

GNPs in the 15-20 nm size range have attracted attention for the fabrication of smart sensing devices in biomedical sciences as diagnostic tools.

The surface fictionalization of gold nanoparticles could increase antibody-antigen reaction, which further amplifies the signaling immune assay.

Due to all the above advantages, GNPs were used in the development of lateral flow assay which is a one-step on-site screening test for analysis.

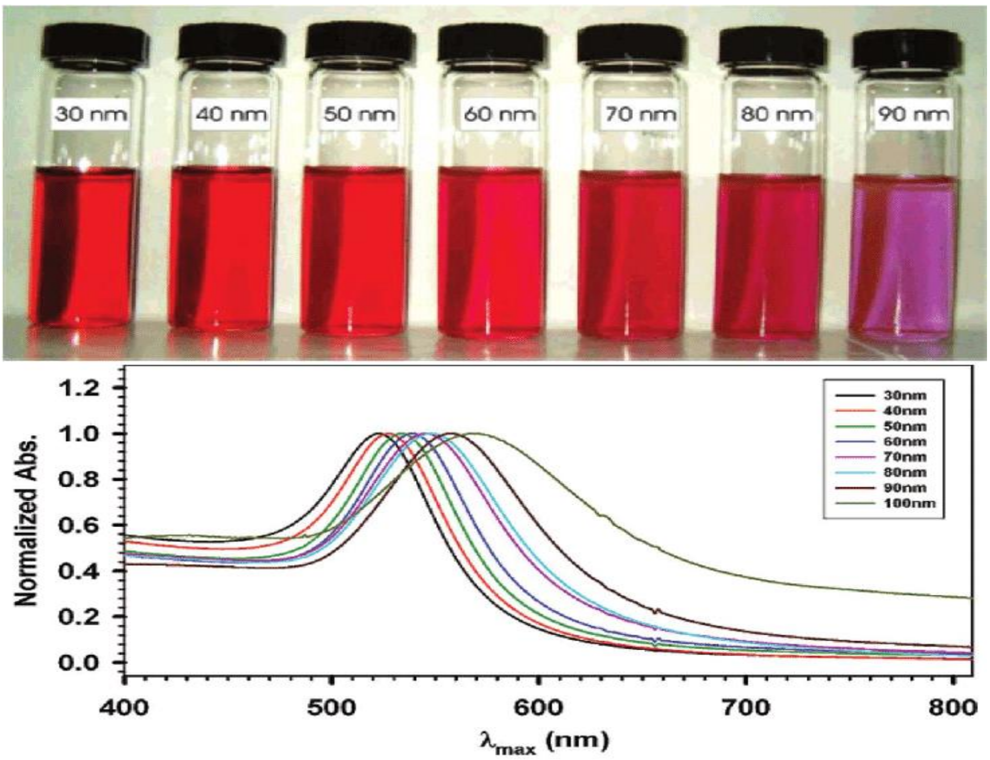

Fig. 1. Gold nanoparticles of different sizes

Synthesis route of nanoparticles

Chemical, physical, and biological processes are the most common methods for producing nanoparticles. Although the chemical path to nanoparticle production is a rapid procedure that yields a huge number of nanoparticles, however, hazardous compounds are utilized for stabilizing and capping nanoparticles, resulting in an unfriendly environment. For the synthesis of nanoparticles, the physical process is usually expensive and requires a complicated experimental apparatus. Furthermore, nanoparticles made by chemical and physical processes are not used in pharmaceuticals. Historically, Many biological processes have been discovered to be capable of transforming metal ions into metal nanoparticles.

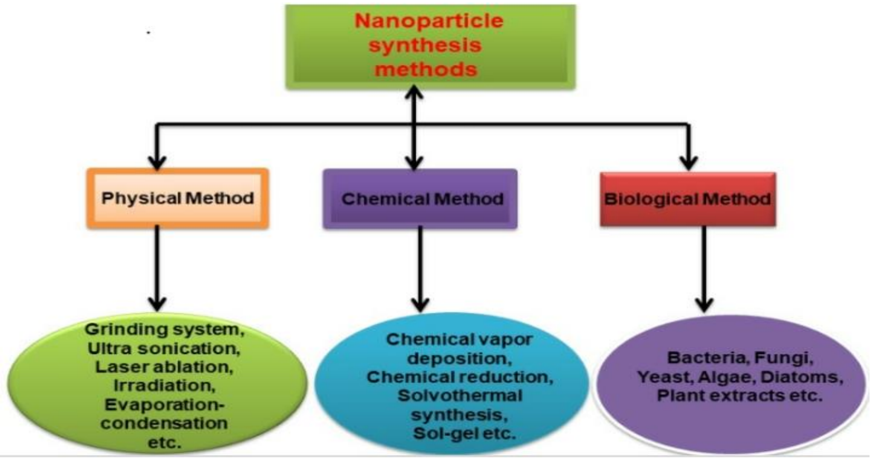

Fig. 2. A figure illustrating the methods of nanoparticles synthesis

Green synthesis of nanoparticles

Green chemistry is a new field that promotes the use of a set of principles aimed at reducing the use and generation of hazardous chemicals. Green approaches, as a result, lessen the environmental impact of industrial labor. Scientists are working to create alternatives to the costly processes and toxic compounds that can be encountered when employing classic physicochemical synthesis methods By lowering metal ions in aqueous solutions, biocompatible metallic NPs can be synthesized by employing environmentally friendly solvents and reagents, minimizing high-energy consumption procedures, and using non-toxic biomolecules such as DNA, proteins, enzymes, carbohydrates, and plant extracts.

Green synthesis employs a clean, safe, cost-effective, and environmentally friendly process of constructing nanomaterials. Microorganisms such as bacteria, yeast, fungi, algal species, and certain plants act as substrates for the green synthesis of nanomaterials. Different active molecules and precursors, such as metal salt, determine the final morphology and size of the nanoparticle. Additionally, green synthesis provides nanomaterial benefits ranging from antimicrobial properties to natural reducing properties and stabilizing properties. The active molecules of the microorganisms utilized as green synthesis substrates attribute to these properties. [Sivaraj, A.; Kumar, V.; Sunder, R.; Parthasarathy, K.; Kasivelu, G et al. 2020]

Synthesis of Nanoparticles from Algae: In algae, polysaccharides can reduce and stabilize metal nanoparticles. The stabilization provided by polysaccharides relies on the presence of multiple binding sites along the polysaccharide chain to facilitate attachment to the surface of the metal, thereby effectively trapping the metal nanoparticle and conferring significant protection against aggregation and chemical modification. Gold nanoparticles, gold, and silica gold Bio nanocomposites can be synthesized from seaweeds and microalgae such as diatoms (Navicular atomic and Diadesmis gallica). Gold, silver and Au/Ag bimetallic nanoparticles can be synthesized from *Spirulina platensis* (also known as edible blue-green alga) [Schrofel A, et al. 2011]

Synthesis of Nanoparticles from Fungi: Fungi contain enzymes and proteins, which have the capabilities of reducing metal ions into nanoparticles and then behaving as a stabilizer for nanoparticles. Fungi produce a large number of proteins, due to which the conversion of metal salts into metal nanoparticles is very fast. Gold nanoparticles have been synthesized in the presence of the fungus Cylindrocladium floridanum. It was noted that in 7 days, the fungi accumulated face-cantered cubic (FCC) (111)-oriented crystalline gold nanoparticles on the surface of the mycelia. The synthesis of gold nanoparticles was confirmed from the characteristic peak on the Uv-Vis spectrum, which appears at 540 nm in the Uv-Vis region [31]. Gold nanoparticles were also synthesized from Aspergillus niger and were confirmed by their adsorption band which appears at 530 nm. [Narayanan KB and Sakthivel N. et al.2011]

Synthesis of Nanoparticles from Yeast: Yeast strains possess more benefits over bacteria because of their mass production of NPs and easy to control yeasts in laboratory circumstances, the synthesis of numerous enzymes, and rapid growth with the use of simple nutrients The incubation of Yarrowia lipolytic cells was done with changed concentrations of chloroauric acid and formed cell-related gold NPs and nanoplates.

Synthesis of Nanoparticles from Bacteria: Bacteria possess a remarkable ability to reduce heavy metal ions and are one of the best candidates for nanoparticle synthesis. It was reported that ferric ions can be reduced to the ferrous state by Thiobacillus ferrooxidans, T. thiooxidans, and Sulphurous acidocaldarius when growing on elemental sulfur as an energy source.

In a recent study, pure gold nanoparticles were produced by the bacterium Delftia acidovorans in which the production of a small non-ribosomal peptide, delftibactin was responsible for generating the gold nanoparticles [Johnston CW, et al.2013].

The extracellular formation of gold nanoparticles of 10-20 nm size was synthesized by the bacterium Rhodo Pseudomonas capsulate. These nanoparticles were synthesized by an NADH-Dependant Reductase [He S, et al.2007].

Synthesis of nanoparticles from plant extract

Plants can accumulate heavy metals in many regions of their bodies. As a result, biosynthesis approaches using plant extracts have attracted considerable attention as a simple, efficient, cost-effective, and practical way for nanoparticle production, as well as an excellent alternative to traditional preparation methods. In a "one-pot" synthesis procedure, a variety of plants can be used to decrease and stabilize metallic nanoparticles. To further investigate the many applications of metal/metal oxide nanoparticles prepared by plant leaf extracts, many researchers have used a green manufacturing approach.

The extract is combined with metal precursor solutions at varied reaction conditions for nanoparticle production mediated by plant leaf extract [Mittal

AK, Chisti Y, Banerjee UC, et al. 2013]

The pace of nanoparticle creation, as well as their yield and stability, are admittedly controlled by the parameters influencing the circumstances of the plant leaf extract (such as varieties of phytochemicals, phytochemical concentration, metal salt concentration, pH, and temperature).

Plant leaf extracts contain phytochemicals that have an extraordinary ability to decrease metal ions in a much shorter time than fungus and bacteria, which require a longer incubation time. As a result, plant leaf extracts are thought to be a good and safe source for metal and metal oxide nanoparticle production. Furthermore, plant leaf extract serves a dual purpose in the nanoparticle's creation process, acting as both a reducing and stabilizing agent to enable nanoparticle synthesis [Malik P, Shankar R, Malik V, et al.2014].

The content of the plant leaf extract is also significant in nanoparticle formation; for example, various plants have variable phytochemical concentration levels. Flavones, terpenoids, sugars, ketones, aldehydes, carboxylic acids, and amides are the primary phytochemicals found in plants [Li X, Xu H, Chen ZS, Chen G., et al. 2011].

In general, there are three phases of metallic nanoparticle synthesis from plant extracts: (1) the activation phase (bioreduction of metal ions/salts and nucleation process of the reduced metal ions), (2) the growth phase (spontaneous combination of tiny particles with greater ones) via a process acknowledged as Ostwald ripening, and (3) the last one is termination phase (defining the final shape of the nanoparticles)

Leaves, bark, stem, shoots, seeds, latex, secondary metabolites, roots, twigs, peels, fruits, seedlings, essential oils, and tissues are all examples of plant parts or products that can be extracted. Polyphenols, flavonoids, carbohydrates, enzymes, and proteins are abundant in them. These phytochemicals are isolated and used directly in the extracellular production of metallic NPs as reducing and stabilizing agents, substituting potentially dangerous compounds like sodium borohydride ($NaBH_4$).

Due to the large diversity of phytoconstituents found in the extracts, the particular mechanism for this phenomenon has yet to be understood. Although polyphenols, organic acids, and proteins are thought to be the principal reducing agents, the diverse phytochemicals are thought to operate together. In general, this strategy is a cost-effective option

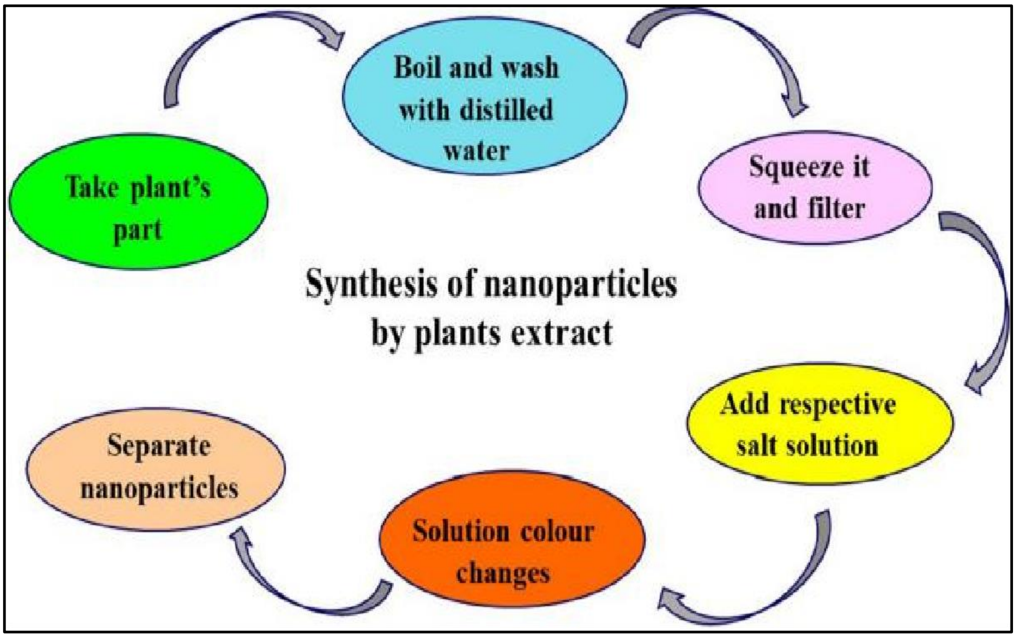

Fig. 3. Synthesis of nanoparticles from plant extract

Antimicrobial activity of nanoparticles

Various studies have been carried out to ameliorate antimicrobial functions because of the growing microbial resistance toward common antiseptics and antibiotics. According to in vitro antimicrobial studies, the metallic nanoparticles effectively obstruct several microbial species. The antimicrobial effectiveness of the metallic nanoparticles depends upon two important parameters: (a) the material employed for the synthesis of the nanoparticles and (b) their particle size.

Over time, microbial resistance to antimicrobial drugs has become gradually raised and is, therefore, a considerable threat to public health. For instance, antimicrobial drug-resistant bacteria contain methicillin-resistant, sulphonamide-resistant, penicillin-resistant, and vancomycin-resistant properties.

The emergence of antibiotic-resistant pathogens has become a serious health issue and thus, numerous studies have been reported to improve the current antimicrobial therapies. It is known that over 70% of bacterial infections are resistant to one or more of the antibiotics that are generally used to eradicate the infection.

The development of new and effective antimicrobial agents seems to be of paramount importance. The antimicrobial activity of metals such as silver (Ag), copper (Cu), gold (Au), titanium (Ti), and zinc (Zn), each having various properties, potencies, and spectra of activity, has been known and applied for centuries.

The application of nanomaterials in drug delivery systems has been investigated for more than twenty years bringing about the innovation of dosage forms with improved therapeutic effects and physicochemical characteristics.

Several types of nanoparticles and their derivatives have received great attention for their potential antimicrobial effects. Metal nanoparticles such as Ag, silver oxide (Ag_2O), titanium dioxide (TiO_2), silicon (Si), copper oxide (CuO), zinc oxide (ZnO), Au, calcium oxide (CaO), and (MgO) were identified to exhibit antimicrobial activity. In vitro studies revealed that metal nanoparticles inhibited several microbial species.

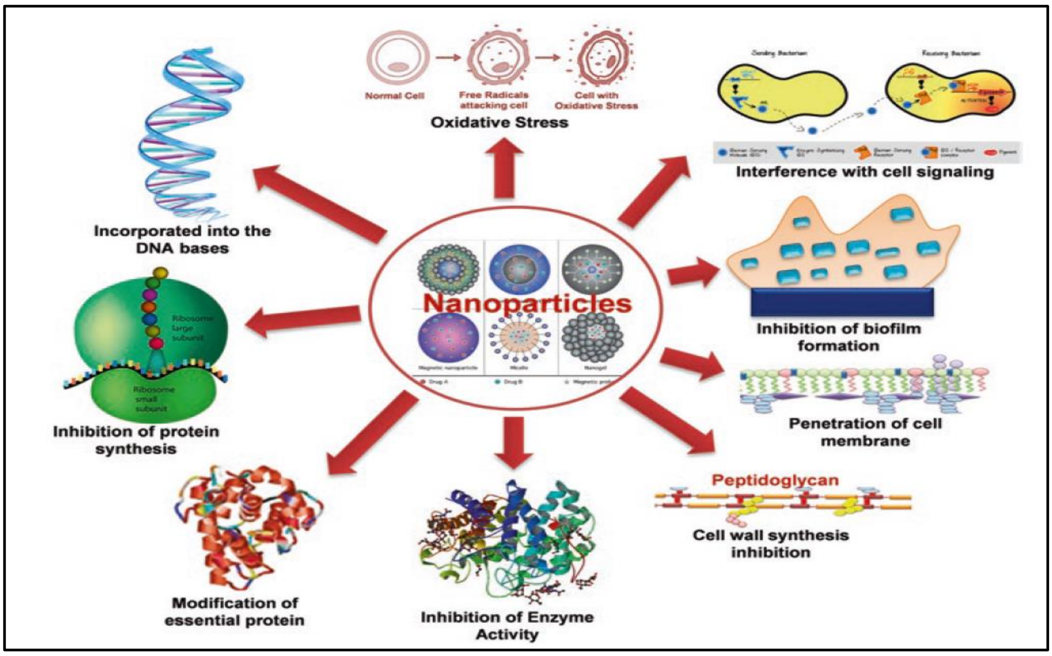

Fig. 4. Antimicrobial activity of nanoparticles

Applications of nanoparticlesTissue engineering

Natural bone surface quite often contains features that are about 100 nm across. If the surface of an artificial bone implant were left smooth, the body would try to reject it. Because of that smooth surface is likely to cause the production of fibrous tissue covering the surface of the implant. This layer reduces the bone-implant contact, which may result in the loosening of the implant and further inflammation. It was demonstrated that by creating nano-sized features on the surface of the hip or knee prosthesis one could reduce the chances of rejection as well as stimulate the production of osteoblasts. The osteoblasts are the cells responsible for the growth of the bone matrix and are found on the advancing surface of the developing bone.

The effect was demonstrated with polymeric, ceramic, and, more recently, metal materials. More than 90% of the human bone cells from suspension adhered to the nanostructured metal surface, but only 50% in the control sample. In the end, this finding would allow to design of more durable and longer-lasting hip or knee replacements and reduce the chances of the implant getting loose.

Titanium is a well-known bone repairing material widely used in orthopedics and dentistry. It has a high fracture resistance, ductility, and weight-to-strength ratio. Unfortunately, it suffers from a lack of bioactivity, as it does not support cell adhesion and growth well. Apatite coatings are known to be bioactive and bond to the bone. Hence, several techniques were used in the past to produce an appetite coating on titanium. Those coatings suffer from thickness non-uniformity, poor adhesion, and low mechanical strength. In addition, a stable porous structure is required to support the nutrients transported through the cell growth.

It was shown that using a biomimetic approach a slow growth of nanostructured apatite film from the simulated body fluid resulted in the formation of a strongly adherent, uniform nanoporous layer. The layer was found to be built of 60 nm crystallites and possess a stable nanoporous structure and bioactivity.

A real bone is a nanocomposite material, composed of hydroxyapatite crystallites in the organic matrix, which is mainly composed of collagen. Thanks to that, the bone is mechanically tough and, at the same time, plastic, so it can recover from mechanical damage. The actual nanoscale mechanism leading to this useful combination of properties is still debated.

An artificial hybrid material was prepared from 15–18 nm ceramic nanoparticles and poly (methyl methacrylate) copolymer. Using the tribology approach, a viscoelastic behavior (healing) of the human teeth was demonstrated. An investigated hybrid material, deposited as a coating on the tooth surface, improved scratch resistance as well as possessed a healing behavior similar to that of the tooth.

Cancer therapy

Photodynamic cancer therapy is based on the destruction of the cancer cells by laser-generated atomic oxygen, which is cytotoxic. A greater quantity of a special dye that is used to generate the atomic oxygen is taken in by the cancer cells when compared with healthy tissue. Hence, only the cancer cells are destroyed and then exposed to laser radiation. Unfortunately, the remaining dye molecules migrate to the skin and the eyes and make the patient very sensitive to daylight exposure. This effect can last for up to six weeks.

To avoid this side effect, the hydrophobic version of the dye molecule was enclosed inside a porous nanoparticle. The dye stayed trapped inside the Ormosil nanoparticle and did not spread to the other parts of the body. At the same time, its oxygen-generating ability has not been affected and the pore size of about 1 nm freely allowed for the oxygen to diffuse out.

Protein detection

Proteins are an important part of the cell's language, machinery, and structure, and understanding their functionalities is extremely important for further progress in human well-being. Gold nanoparticles are widely used in immunohistochemistry to identify protein-protein interaction. However, the multiple simultaneous detection capabilities of this technique are fairly limited. Surface-enhanced Raman scattering spectroscopy is a well-established technique for the detection and identification of single dye molecules.

Combining both methods in a single nanoparticle probe one can drastically improve the multiplexing capabilities of protein probes. The group of Prof. Mirkin has designed a sophisticated multifunctional probe that is built around a 13 nm gold nanoparticle. The nanoparticles are coated with hydrophilic oligonucleotides containing a Raman dye at one end and terminally capped with a small molecule recognition element (e.g. biotin). Moreover, this molecule is catalytically active and will be coated with silver in the solution of Ag (I) and hydroquinone. After the probe is attached to a small molecule or an antigen it is designed to detect, the substrate is exposed to silver and hydroquinone solution. A silver-plating is happening close to the Raman dye, which allows for dye signature detection with a standard Raman microscope. Apart from being able to recognize small molecules, this probe can be modified to contain antibodies on the surface to recognize proteins. When tested in the protein array format against both small molecules and proteins, the probe has shown no cross-reactivity.

In manufacturing and material

Nanocrystalline materials provide very interesting substances for material science since their properties deviate from respective bulk materials in a size-dependent manner. Manufacture NPs display physicochemical characteristics that induce unique electrical, mechanical, optical, and imaging properties that are extremely looked for in certain applications within the medical, commercial, and ecological sectors (Dong et al., 2014, Ma, 2003, Todescato et al., 2016).

NPs focus on the characterization, designing, and engineering of biological as well as non-biological structures < 100 nm, which show unique and novel functional properties. The potential benefits of nanotechnology have been documented by many manufacturers at high and low levels and marketable products are already being mass-produced such as in the microelectronics, aerospace, and pharmaceutical industries.

Among the nanotechnology consumer products to date, health fitness products form the largest category, followed by the electronic and computer category as well as the home and garden category. Nanotechnology has been touted as the next revolution in many industries including food processing and packing. Resonant energy transfer (RET) systems consisting of organic dye molecules and noble me have recently gamed considerable interest in biophotonics as well as in material science (Lei et al., 2015). The presence of NPs in commercially available products is becoming more common.

Metal NPs such as noble metals, including Au and Ag have many colors in the visible region based on the plasmon resonance, which is due to collective oscillations of the electrons at the surface of NPs. The resonance wavelength strong depends on the size and shape of NPs, the interparticle distance, and the dielectric property of the surrounding medium. The unique plasmon absorbance features of these noble metals NPs have been exploited for a wide variety of applications including chemical sensors and biosensors.

In environment

The increasing area of engineered NPs in industrial and household applications leads to the release of such materials into the environment. Assessing the risk of these NPs in the environment requires an understanding of their mobility, reactivity, Ecotoxicity, and persistency.

The engineering material applications can increase the concentration of NPs in groundwater and soil which presents the most significant exposure avenues for assessing environmental risks.

Due to the high surface-to-mass ratio natural NPs play an important role in the solid/water partitioning of contaminants that can be absorbed to the surface of NPs, co-precipitated during the formation of natural NPs, or trapped by aggregation of NPs which had contaminants adsorbed to their surface. The interaction of contaminants with NPs is dependent on the NPs' characteristics, such as size, composition, morphology, porosity, aggregation/disaggregation, and aggregate structure. The luminophores are not safe in the environment and are protected from environmental oxygen when they are doped inside the silica network.

Most environmental applications of nanotechnology fall into three categories:

1. Environmentally benign sustainable products (e.g. green chemistry or pollution prevention).
2. Remediation of materials contaminated with hazardous substances and
3. Sensors for environmental stages.

The removal of heavy metals such as mercury, lead, thallium, cadmium, and arsenic from natural water has attracted considerable attention because of their adverse effects on environmental and human health. Superparamagnetic iron oxide NPs are an effective sorbent material for this toxic soft material. So, no measurements of engineered NPs in the environment have been available due to the absence of analytical methods, able to quantify trace concentration of NPs.

Photodegradation by NPs is also a very common practice and many nanomaterials are utilized for this purpose. Rogozea et al. used NiO/ZnO NPs modified silica in the tandem fashion for photodegradation purposes. The high surface area of NPs due to their very small size (<10 nm), facilitated the efficient photodegradation reaction).

Application in electronics

There has been growing interest in the development of printed electronics in the last few years because printed electronics offer attractive to traditional silicon techniques and the potential for low-cost, large-area electronics for flexible displays, and sensors. Printed electronics with various functional inks containing NPs such as metallic NPs, organic electronic molecules, CNTs, and ceramics NPs have been expected to flow rapidly as a mass production process for new types of electronic equipment (Kosmala et al., 2011).

Unique structural, optical, and electrical properties of dimensional semiconductors and metals make them the key structural block for a new generation of electronic, sensors and photonic materials.

A good example of the synergism between scientific discovery and technological development is the electronic industry, where discoveries of new semiconducting materials resulted in the revolution from vacuumed tubes to diodes and transistors, and eventually to miniature chips).

The important characteristics of NPs are facile manipulation and reversible assembly which allow for the possibility of incorporation of NPs in electric, electronic or optical devices such as “bottom-up” or “self-assembly” approaches are the benchmark of nanotechnology.

In energy harvesting

Due to their nonrenewable nature, recent research has cautioned us about the limitations and shortage of fossil fuels in the next years. As a result, scientists are altering their research tactics to develop low-cost renewable energy from readily available resources. Because of its huge surface area, optical characteristics, and catalytic nature, they discovered that NPs are the greatest contender for this purpose. NPs are commonly utilized to generate energy from photoelectrochemical (PEC) and electrochemical water splitting, particularly in photocatalytic applications.

NPs are also used in energy storage applications to store energy in various ways at the nanoscale.

Nanogenerators have recently been developed that can transform mechanical energy into electricity utilizing piezoelectric technology, which is a novel way of energy generation.

Drug delivery

Drug delivery and related pharmaceutical development in the context of nanomedicine should be viewed as science and technology of nanometer-scale complex systems (10–1000 nm), consisting of at least two components, one of which is a pharmaceutically active ingredient although nanoparticle formulations of the drug itself are also possible.

The whole system leads to a special function related to treating, preventing, or diagnosing diseases sometimes called smart drugs or theragnostic.

The primary goals for research of nano-bio-technologies in drug delivery include:

❖ More specific drug targeting and delivery,

❖ Reduction in toxicity while maintaining therapeutic effects,

❖ Greater safety and biocompatibility, and

❖ Faster development of new safe medicines.

The main issues in the search for appropriate carriers as drug delivery systems pertain to the following topics that are basic prerequisites for the design of new materials. They comprise knowledge on (i) drug incorporation and release, (ii) formulation stability and shelf life (iii) biocompatibility, (iv) biodistribution and targeting, and (v) functionality. In addition, when used solely as a carrier the possible adverse effects of residual material after the drug delivery should be considered as well. In this respect biodegradable nanoparticles with a limited life span as long as therapeutically needed would be optimal.

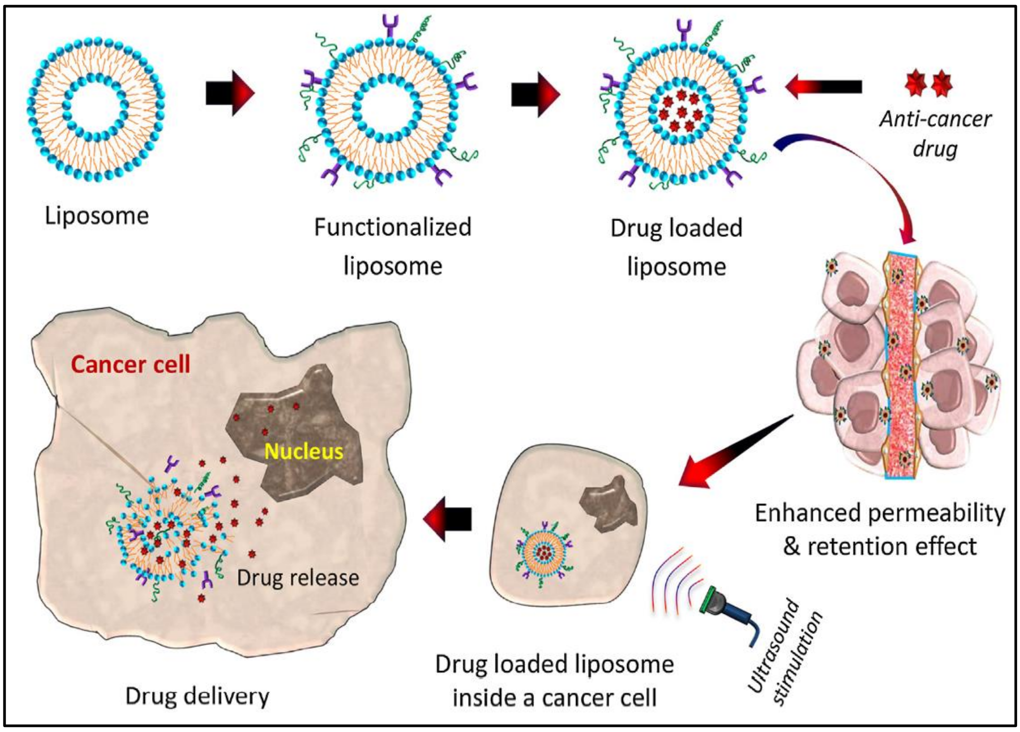

Fig. 5. A diagrammatic presentation of drug delivery throughnanoparticles

Morpankhi (*Platycladius Orientalis)*

Morpankhi is a plant that is found in every small garden in India. Belonging to the cypress family, it is a densely branched evergreen conifer that can become 50 ft tall with a spread of 20. However, it is commonly grown as a smaller, bushier shrub. It tends to have several too many stems but can be trimmed to a single leader stem creating a treelike form. The overall shape is conical, with the crown becoming more irregular and spreading with age. The bark is rusty-brown and fibrous. The numerous slender ascending branches support shoots that spread out in flat, vertical planes. The leaves are like little scales overlapping and tightly packed on the shoots. The cones are 15-25 mm long, green ripening brown in about 8 months from pollination, and have 6-12 thick scales arranged in opposite pairs. Morpankhi is native to China and Korea but is cultivated as an ornamental plant the world over.

Thuja orientalis (Commonly Morpankhi, Family Cupressaceae) is a genus of coniferous trees. T. Orientalis is an evergreen, monoecious tree or shrub growing to 10-60 feet tall. The shoot is flat, leaves are scale-like. The leaves are arranged in a flattened fan-shaped growing with resin glands. Their leaves contain essential oils used to treat fungus infections, cancer, moles, and parasitic worms. The essential oil derived from the leaves is toxic. α-thujone is useful as an insecticide and an anthelminthic agent for the treatment of parasitic worms. However, α-thujone is a toxic substance that disrupts neurological signals in the brain. Ingestion of the essential oils of Thuja leaves can cause death. Seed with a pair of narrow lateral wings, seedlings produce 2 cotyledons. The wood is light, soft, and aromatic. Thuja poles are also often used to make fence posts and rails. The wood of Thuja is commonly used for guitar soundboards. It is used as a medicinal plant in various forms of traditional medicines like folk medicine, homeopathy, and treatment of bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea, and rheumatism. Oil of Thuja contains thujone which has been studied for its GABA (gamma-aminobutyric acid) receptor antagonistic, with potentially lethal properties. A yellow dye is obtained from the young branches. Thuja is also occasionally used for treating diseases of the skin, blood, gastrointestinal tract, kidney, brain, warty excrescences, and spongy tumors.

Fig. 6. Platycladious Orientalis

Chemical constituents

Thuja orientalis leaves contain rhodoxanthin, amentoflavone, hinokiflavone, quercetin, myricetin, carotene, xanthophylls, and ascorbic acid. The fruit and roots are strongly aromatic. Distillation of the dried roots yields an essential oil having the following properties. Thuja Orientalis leaves contain rhodoxanthin, amentoflavone, hinokiflavone, quercetin, myricetin, carotene, xanthophylls, and ascorbic acid. The fruit and roots are strongly aromatic. Distillation of the dried roots yields an essential oil having the following properties- saturated acids, 6.10%. The heartwood contains aroma- dendrin, taxifolin, widdrene, cedrol, thujopsadiene, dehydro-α-curcumene, β- isobiotol and Curcumenether. It also contains an essential oil C is a complex blend of Sesquiterpene hydrocarbons (cuparenes) 40; alcohols (Cedrol, windrow, cuparenols) 50; monoterpene acids 19 and 28 compounds have been identified in the volatile oils of the fruit and leaf, respectively, while the fruit oil contained α-pinene (52.4%), 3-carene (14.2%), α-cedrol (6.5%) and phellandrene (5.1%), the leaf oil contained α-pinene (21.9%), α-cedrol (20.3%), 3-carene (10.5%) and limonene (7.2%) as the main components. Thuja orientalis leaves contain rhodoxanthin, amentoflavone, isoflavone, quercetin, myricetin, carotene, xanthophylls, and ascorbic acid. The fruit and roots are strongly aromatic. Distillation of the dried roots yields an essential oil The composition of the oil is as follows: a new bicyclic sesquiterpene 51.10; l-borneol, 17.10; bornyl acetate, 9.1; α-thujone and camphor, 5.6; and new sesquiterpenoid alcohol The seed yields fatty oil having the following

composition palmitic 5.28, stearic, 7.3; C18 unsaturated acids, 1829 (linolenic, 44.6%); and C20 un- saturated acids, 6.10%. The heartwood contains aroma-dendrin, taxifolin, widener, cedrol, thujopsadiene, dehydro-α-curcumene, β-isobiotol and Curcumenether. It also contains an essential oil C is a complex blend of Sesquiterpene hydrocarbons (cuparenes) 40; alcohols (Cedrol, windrow, cuparenols) 50; monoterpene acids [Nickavar et al.,]
28 compounds have been identified in the volatile oils of the fruit and leaf, respectively, while the fruit oil containedα-pinene (52.4%), 3-carene (14.2%), α-cedrol (6.5%), and phellandrene (5.1%), the leaf oil contained α-pinene (21.9%), α-cedrol (20.3%), 3-carene (10.5%) and limonene (7.2%) as the main components.

Antibacterial activity

Thuja orientalis contain large amounts of three substances (alpha, beta, and gamma thujaplicin) that in low concentration would serve as chelators for Salmonella typhimurium [18]. *T. Orientalis* was very effective in inhibiting the growth of serotypes c and d of Salmonella mutant (MIC less than or equal to 2.0-7.8 mg/ml).

Antifungal activity

The essential oil showed antifungal activity in the inhibition zone against Alternaria alternate and *Currularia lunatic* in a direct bioautography assay by lipophilic leaf extract of *T. Orientalis*. Best bioactive components (Rf = 0.80) were observed and noted for antifungal activity. It produced an inhibition zone of 30 and 22 mm in diameter against *A. alternata* and *C. Lunata*, respectively.

Reports of antifungal activity of aqueous leaf extract of *T. orientalis* against *Curvularia lunata*. The essential oils from leaves, twigs, and stems of large trees and shrub-like trees of Thuja sutchuenensis were extracted by hydrodistillation and supercritical fluid extraction and analyzed by GC and GC-

MS. The essential oils exhibited a certain degree of antifungal activity against six strains of human pathogenic fungi.

Antiviral activity

The chemical composition of the essential oil of T. orientalis was determined by GC/MS analysis. Essential oils have been evaluated for their inhibitory activity against Severe Acute Respiratory Syndrome Coronavirus (SARS Coronavirus) and Herpes Simplex Virus Type-1 (HSV-1) replication in vitro by visually scoring the virus-induced cytopathogenic effect post-infection. Several types of research have demonstrated that allopathic extracts of T. orientalis could be used as strong antiviral agents against plant and animal viruses.

Inflammatory activity

Vascular inflammation is involved in the inhibition and progression of cardiovascular disease including atherosclerosis. The anti-vascular inflammatory activity of an aqueous extract of T. orientalis (ATO) and its possible mechanisms was investigated in human umbilical vein endothelial cells.

Preincubation of ATO inhibited tumor necrosis factor and also inhibited U937 monocytes adhesion to HUVECs stimulated by tumor necrosis factor (TNF) suggesting that it may inhibit the binding of monocytes to the endothelium. Furthermore, ATO significantly inhibited the TNF-induced production of intracellular reactive oxygen species (ROS). Overall, ATO has an anti-inflammatory activity which is at least in part, is due to the decrease in the TNF-induced endothelial adhesion to monocytes by inhibiting intracellular ROS production, NF-κB activation, and cell adhesion molecule in HUVECs

Anticancer activity

The strong 5α a-reductase inhibitor is extracted and fractionated from T. orientalis and purified as diterpenes in the isolated form [30]. The inhibitors are used either on their own or as active ingredients of therapeutics in the treatment of diseases caused by the overactivity of 5αa-reductase or the hypersecretion of androgens, such as male baldness, androgenetic alopecia, hirsutism, acne, prostatomegaly and cancer of the prostate.

Larvicidal activity

Larvicidal activities of *T. orientalis* oil against 4th instar larvae of Aedes aegypti and Culex pippin's pallens have been observed.

The larvicidal activity of T. orientalis leaf oil was higher than those of stem, fruit, and seed oils. Essential oils of leaves and fruits of T. orientalis at 400 ppm caused 100% and 71.6% mortalities against A. aegypti.

The larvicidal activity was observed from various age classes (I-III) and found strong mortality in the age class II of T. orientalis against Aedes aegypti and Culex pipiens pallens larvae. Leaf part and age class II of T. orientalis has strong larvicidal activity against Aedes aegypti and Culex pipiens pallens. Leaf oil of T. orientalis shows natural larvicides against Aedes aegypti and Culex pipiens pallens

Insecticidal activity

Insecticidal Activity Leaf extracts of *T. orientalis* show a repellent activity against Chilo partellus. *T. orientalis* ether extract (68.63%), and acetone extracts (67.51%) have sufficient repellent action. Foliar application of a semi-solid crude extract of *T. orientalis* on maize was very effective against Chilo partellus.

Molluscicidal activity

The ethanol extract of *T. orientalis* leaf (24 h LC50- 32.74 mg/l) and column purified fraction (24 h LC50- 29.25 mg/l) were potent molluscicides against Lymnaea acuminate. Thujone (24 h LC50- 08.09 mg/l) was identified as an active molluscicidal component in T. orientalis. The molluscicidal activity of leaf/fruit of *Thuja orientalis* and their active components/column purified fraction with synergist Piperonyl butoxide (PB) and MGK264 (ENT 8184) was studied in binary combination (1:5) against *L acuminate*. A combination of *T. orientalis* leaf/ thujone or fruit powder/ column extract of T. orientalis fruit with PB or MGK-264 indicates synergized toxicity up to 189.02 times.

Objectives

Objectives

- Synthesis of gold nanoparticles by using Thuja orientalis leaf aqueous extract.
- Characterization of synthesized Nanoparticles by UV-Visible spectroscopy, DLS, Zeta potential, FTIR, and TEM
- To check the antibacterial potency of synthesized TO-GNPs.

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 afterthey were prepared. Microbiological media and ingredients were purchased from Himedia, India. All solvents and chemicals were of analytical grade andused as obtained from Merck and Sigma Aldrich (St. Louis, MO, USA)

Method

Preparation of Thuja orientalis leaf extract-

Fresh *Thuja orientalis* was collected from integral university Kursi Road (Lucknow, India) and used for the preparation of aq. extract. The bark was peeled off from the plant and was cleaned with running tap water, freeze-dried, and 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 $HAuCl_4$ salt in PBS buffer (pH was 7.2 and it was filtered by 0.2µm filter) and 0.48ml of freshly prepared Thuja orientalis 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 (III) 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 Nanoparticle

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 stepswere 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

Result

Fig. 7. Synthesized TO-GNPs

Characterization of Gold Nanoparticles: Characterization is done by using UV- visible spectroscopy.

UV- vis Spectroscopy-

The absorption spectra of AuNPs were recorded on Shimadzu Dual Beam Spectrophotometer (model UV–1601PC) in the wavelength range of 200-800 nm in Quartz Cuvette of 1 cm Path Length.

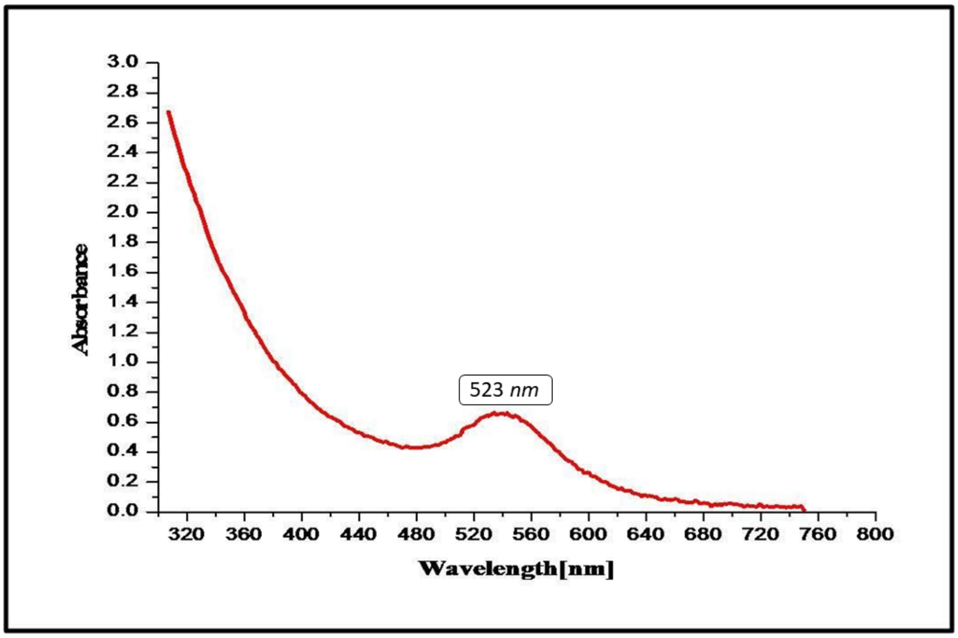

Fig. 8. UV-Vis Spectra of synthesized Gold nanoparticles showing a peak at 523 nm.

UV-visible absorption spectra of gold nanoparticles exhibit a surface Plasmon resonance peak at 523nm the broadness and intensity show that the particle is small in size. If gold nanoparticles are below 2 nm in diameter, this Plasmon peak disappears due to the change in electronic structure that occurs with quantum confinement.

DLS (Dynamic Light Scattering)

The thin electric dipole layer of the solvent adheres to the surface of a dispersed nanoparticle when it moves through a liquid medium; therefore, the hydrodynamic diameter estimated by DLS provides us information about the inorganic core along with coating material and the solvent layer attached to the particle.

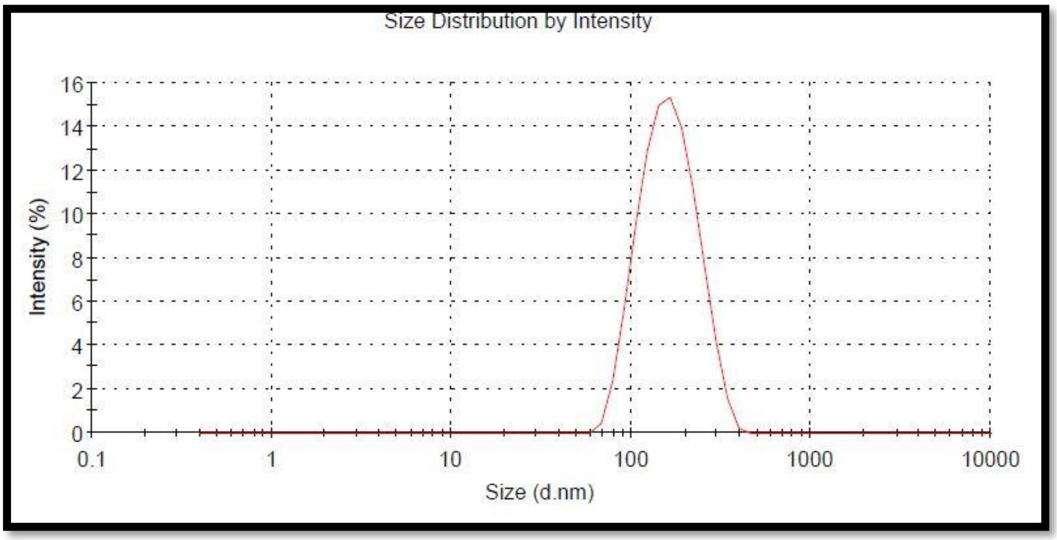

Fig. 9. Size distribution of colloidal AuNPs (size distribution by intensity).

Zeta Potential

The determination of Zeta Potential is considered an effective, simplest, and most straightforward method to predict the stability and understand the surface properties of the nanoparticles. Information concerning the concentration, distribution, exposure, or shielding of charged moieties, ionization, and adsorption could be drawn from the analysis of zeta potential.

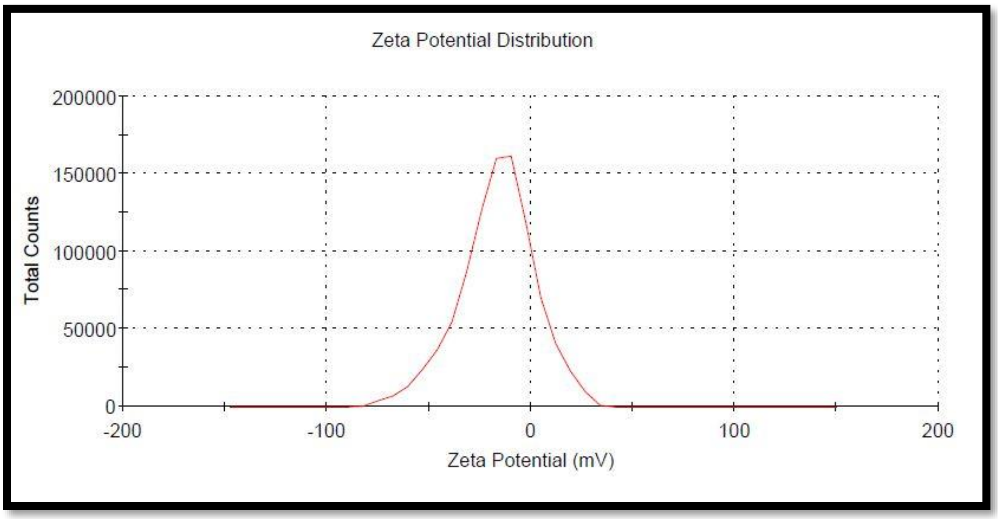

Fig. 10. Zeta potential graph for AuNPs

FTIR

The FTIR analysis of synthesized TO-AuNPs depicts an existing peak focused at 1643.24 cm^{-1}, i.e., uniqueness of amide C=O groups. A medium and wide shoulder for the amide I linkage and amide II band was observed at 1537.02 cm^{-1}. N–H twist and carboxyl stretch in the protein amide bond were found to be responsible for the presence of amides bands I and II that are capped or surface-modified on TO-AuNPs. The N–H stretch vibration peak was observed at 3296.5 cm^{-1}; however, this vibration is susceptible to hydrogen bond strength with no dependence on backbone conformation. Moreover, the alcohol and ether group (C–O–C/C–OH) C–O stretch along with (aliphatic amine) the C–N stretch vibration, showed a peak at 1081.8 cm^{-1}. The alkynes C≡C stretched vibration because numerous secondary metabolites showed a peak at 2127.39 cm^{-1}. Peaks at 3756.013 and 3868.76 cm^{-1} were observed for free (O–H) hydroxyl on the terminus.

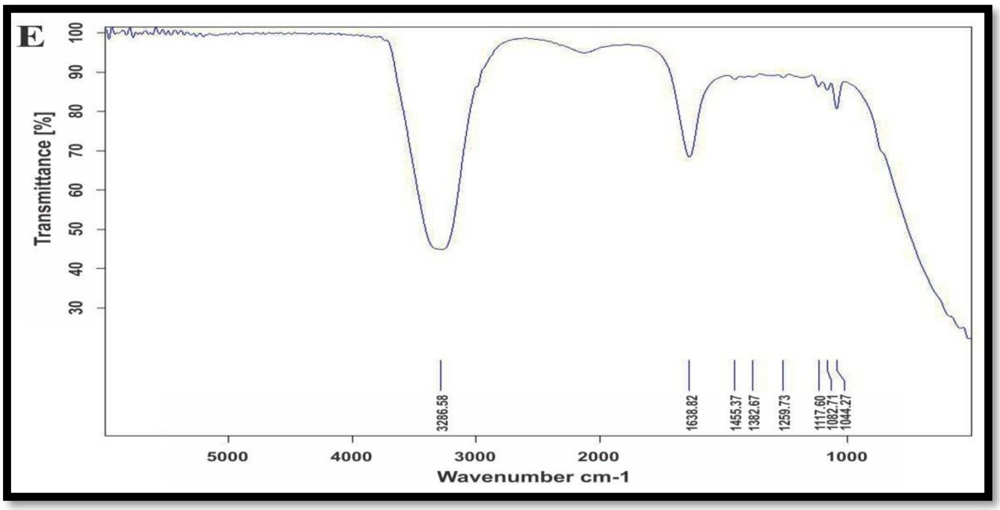

Fig. 11. FTIR spectroscopy.

Transmission electron microscopy

The high-resolution image was acquired using the transmission electron microscope (TEM), which confirmed the average size of TO-AuNPs as 26 ± 2 nm using a Gatan digital micrograph and showed the spherical form of AgNPs. The TEM micrographs did not expose the agglomeration of the as-synthesized AuNPs.

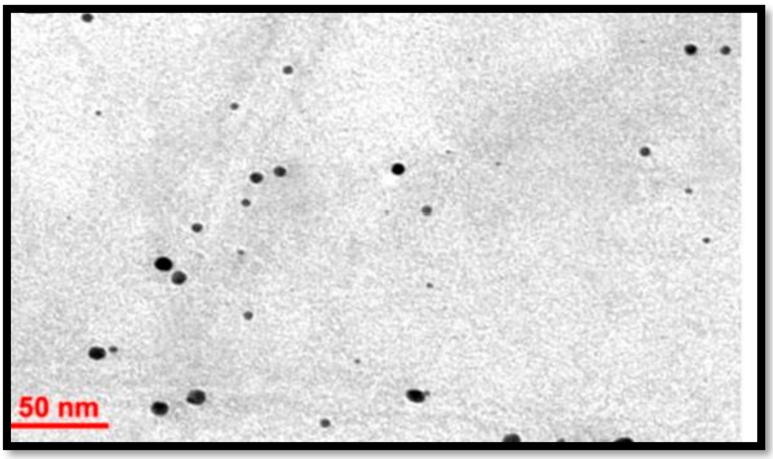

Fig. 12. TEM analysis

Antibacterial activity

The synthesized AuNPs was showing activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilus* causing the zone of inhibition. The antibacterial activities of synthesized gold nanoparticles from *Thuja orientalis* leaf extract against selected bacteria showed in fig. shown below.

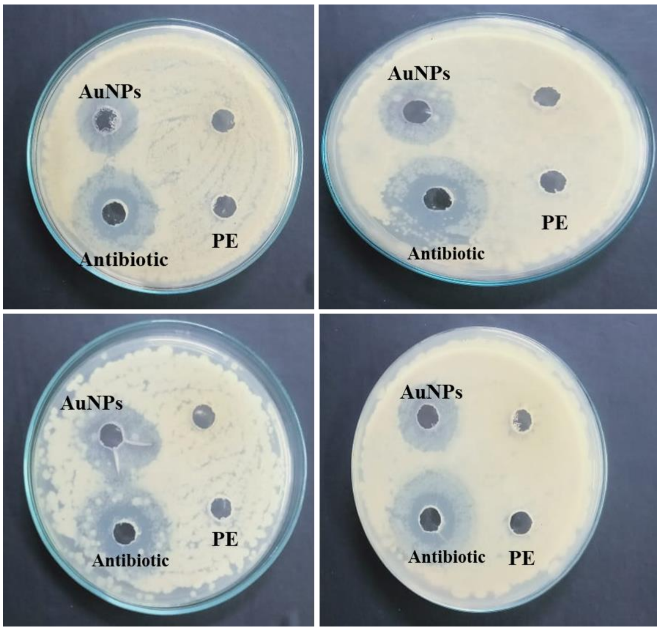

Fig. 13. The synthesized AuNPs showed activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus* by causing a zone of inhibition.

Anti-bacterial analysis

S.No.	**Species**	**Gold Nanoparticle (Zone Of Inhibition in mm)**		
		AuNPs (50µg/well)	**Antibiotic**	**Crude Plant extract**
1.	*Escherichia coli*	**15**	**24**	**7**
2.	*Pseudomonas aeruginosa*	**16**	**26**	**8**
3.	*Staphylococcus aureus*	**11**	**20**	**6**
4.	*Bacillus subtilus*	**10**	**18**	**5**

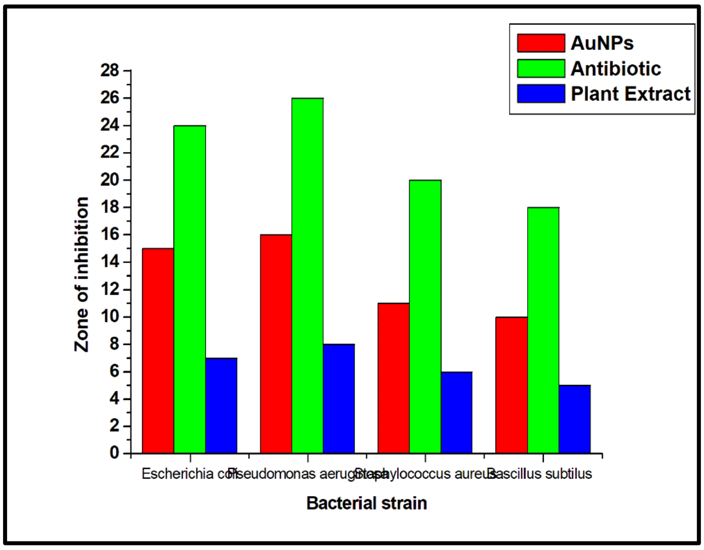

Fig. 14. Table and graph showing zone of inhibition of TO-AuNPs.

The antibacterial studies with TO-AuNPs showed a profound antibacterial effect against both gram-positive and gram-negative strains. The results of the present study suggest that plants and silver in their nano form possess certain constituents with antibacterial properties that may be used as antibacterial agents in new drugs against common bacterial pathogens. The synthesized nanoparticle was active on all the organisms tested. The highest activity against the tested bacteria was obtained on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus* gram-positive and negative bacteria.

MIC (Minimum Inhibitory Concentration)

We found that biogenic AuNPs synthesized by *Thuja orientalis* aqueous leaf extract show strong antibacterial activity against both Gram-positive and Gram-negative pathogenic bacterial strains. The MIC_{50} of TO-AuNPs was evaluated against different pathogenic bacterial strains that included 14.5 µg/ml against *S. aureus*, 8.6 µg/ml against *M. luteus*, 6.063 µg/ml against *E. coli*, and 13.4 µg/ml against *K. pneumoniae*, indicating its broad-spectrum feature. However, we found that TO-AuNPs were more effective against *E. coli* (Gram-negative and *M. luteus* (Gram-positive) than other pathogenic strains. A thick peptidoglycan layer in Gram-positive bacteria prohibited the entry of AuNPs into the cytoplasm, and a higher AuNPs concentration is required to inhibit the growth of Gram-positive than Gram-negative bacteria.

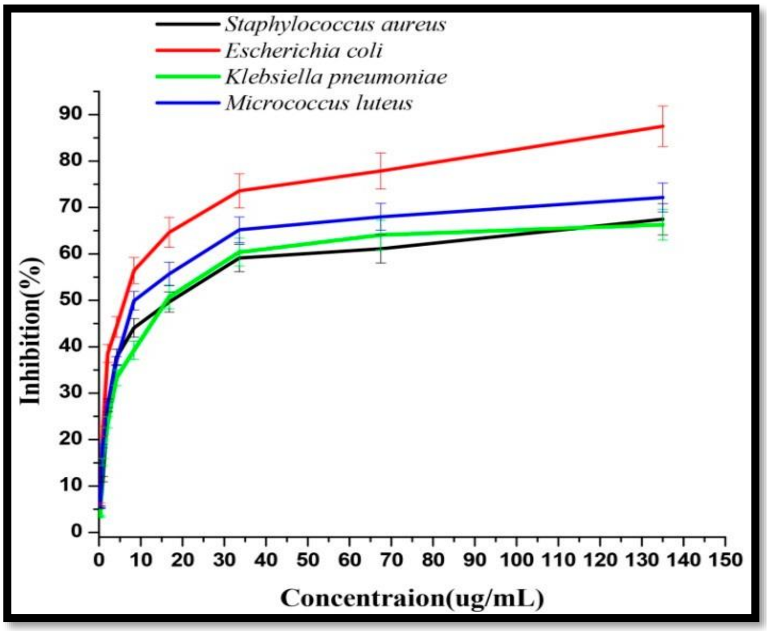

Fig. 15. The antibacterial potential of TO-AuNPs against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, and M. luteus.*

Discussion and conclusion

Discussion

Thuja orientalis (morepankhi) was utilized as a reducing and stabilizing agent in this work. The synthesis of AuNPs is thought to be triggered by *Thuja orientalis* aqueous leaf extract's reducing enzymes and capping agents, such as secondary metabolites, which work together to decrease selenium oxide. 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, *Thuja orientalis leaf* aqueous extract was used for the synthesis of TO-AuNPs. This extract was used as a source for the synthesis of 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 AuNPs was done by UV-visible spectroscopy and peak were found at 523nm, due to the surface plasmon resonance property of Gold Nanoparticles.

Therapeutic Analysis was performed for the AuNPs for antibacterial assay, and multiple drug resistance for different species and found to be positive in most cases. A synthesized gold nanoparticle from *Thuja orientalis leaf* aqueous extract shows broad inhibition against specific bacteria there by 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 *Thuja orientalis* 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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