A DISSERTATION ON

In Vitro Study of *Desmostachya bipinnata* methanolic leaf extract synthesized Gold Nanoparticles

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BY

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Zohra Akhtar**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled ***"In Vitro Study of Desmostachya bipinnata methanolic leaf extract synthesized Gold Nanoparticles"*** 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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IU/DBS/S00192/2022/CIN/221118 **June 2022**

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Zohra Akhtar**, 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 ***"In Vitro Study of Desmostachya bipinnata methanolic leaf extract synthesized Gold Nanoparticles"*** 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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My acknowledgment will be incomplete if I do not mention my parents with whose blessing, I was able to achieve my goal successfully. There are no words to express my feelings toward them. I silently acknowledge my debt to them.

Zohra Akhtar **Date**

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 replacethe high-density flash microchip.

Nanotechnology is used in the medicinal field for high throughput drug

delivery 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, reducingdamage to healthy 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 be used in labs for the early of detection cancercells in bloodstreams. Gold nanorods are being used in combination with infrared rays to sterilize hospital instruments 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 whichactivated 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 causehazardous effects like carcinogenicity, and environmental toxicity. The use of toxic solvents and chemical contaminations limits the use of nanoparticles in various clinical and biomedicalapplications. Hence a need for a cleaner, better, and environmentally friendly approach to thesynthesis 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 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 theirability to execute the purpose for which they were synthesized, as well as any negative impacts.

Review and literature

Nanoparticles

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 asa 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 theaccumulation 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 biodegradablepolymers 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 andbiomolecule tracking [Collier C, et al.1998].

Iron oxide: Iron oxide NPs are studied as passive and active targeting imaging agents becausethey 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 clinicallyapproved 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 variousmodulations dimensionalities: Pokropivyn and Skorokhod classified the nanostructured materials based on their dimensionalities as (1) zero-dimensional nanoparticles (2) 1- 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 varietyof 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 inresearch 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 keyunits 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 developmentof 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 around 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 usedto 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 causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. This

change is related tothe 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 themost frequently used technique for accurate estimation of the particle size and size distributionbased on DLS [De Assis et al., 2008].

Scanning Electron microscopy: Scanning electron microscopy (SEM) is giving morphological examination with direct visualization. The techniques based on electronmicroscopy 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 [Joreset 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 electronbeam 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 itoften 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 deliveryvehicles 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 sensingdevices 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 assaywhich 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 thatyields 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 from chemicals 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.

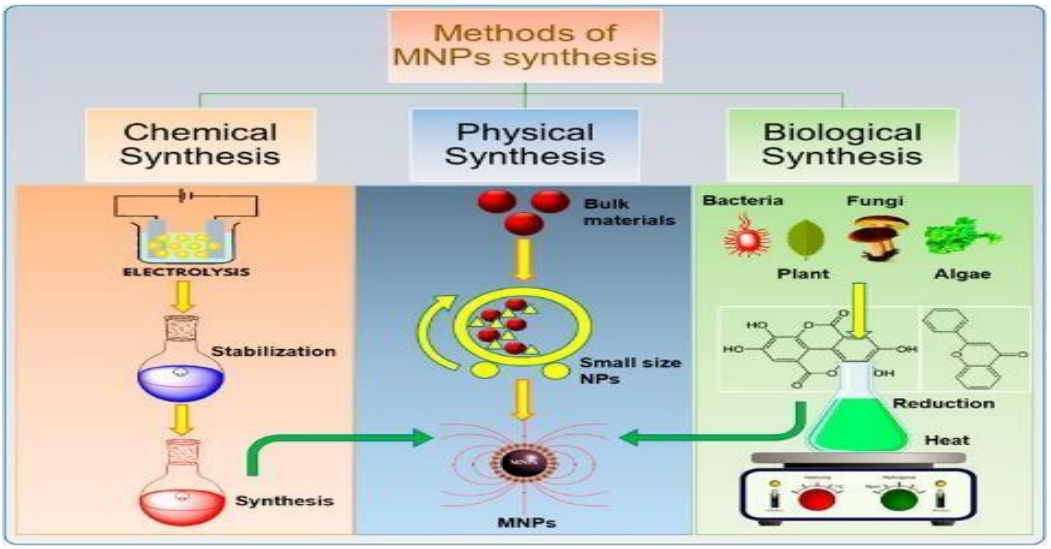

Fig. 2. A figure illustrating the methods of nanoparticle synthesis.

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-toxicbiomolecules 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 fromantimicrobial 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 ofthe metal, thereby effectively trapping the metal.

nanoparticle and conferring significant protection against aggregation and chemical modification. 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 reportedthat 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, etal. 2013].

The pace of nanoparticle creation, as well as their yield and stability, are admittedly controlledby 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 metalions 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 the 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. 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.

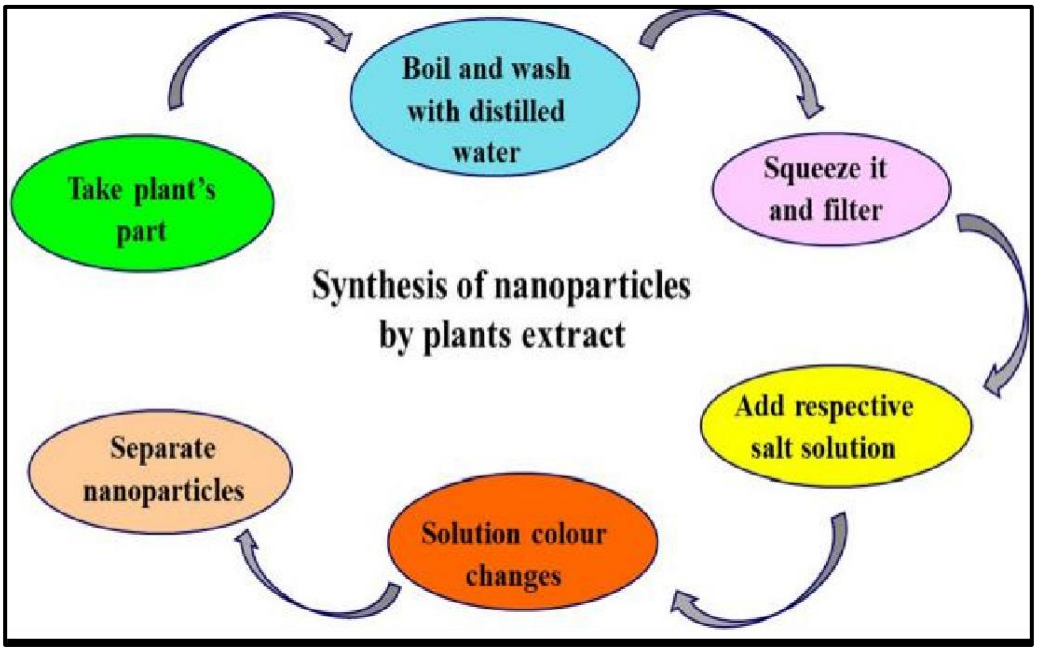

Fig. 3. Synthesis of nanoparticles from plant extract.

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 thatare 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), titaniumdioxide (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.

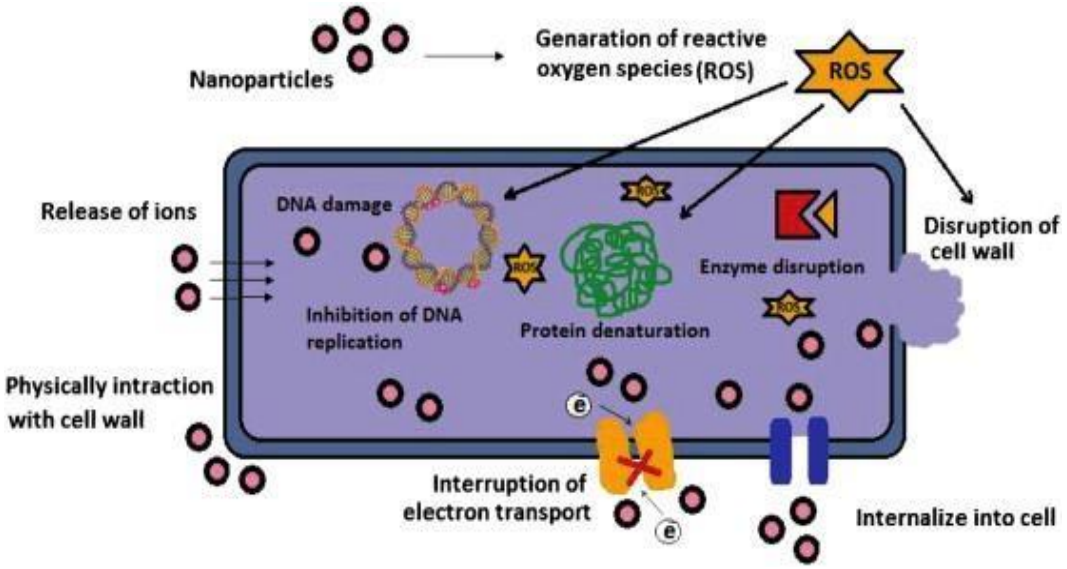

Fig. 4. Antimicrobial activity of nanoparticles.

Applications of nanoparticlesTissue engineering

Natural bone surface quite often contains features that are about 100 *nm* across. If the surfaceof an artificial bone implant were left smooth, the body would try to reject it. Because of thatsmooth 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 onthe 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 growthof 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 metalsurface, but only 50% in the control sample. In the end, this finding would allow to design ofmore 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 suffersfrom 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, the 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 like 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 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 sophisticatedmultifunctional 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 modifiedto 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 theirproperties 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 themedical, 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 have recently gamed considerable interest in bio-photonics 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 regionbased 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 varietyof 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 groundwaterand 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:

Environmentally benign sustainable products (e.g., green chemistry or pollution prevention).

Remediation of materials contaminated with hazardous substances and 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, 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 metalsmake 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 allows 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 non-renewable 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 tacticsto 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 photocatalyticapplications. 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 shouldbe viewed as science and technology of nanometres-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 if therapeutically needed would be optimal.

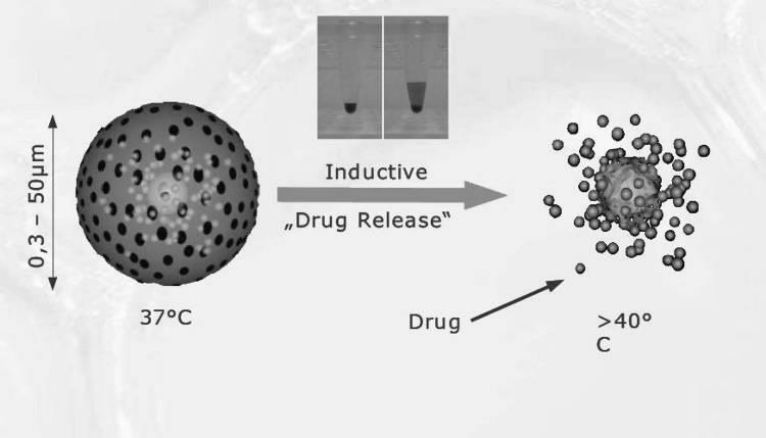

Fig. 5. A diagrammatic presentation of drug delivery through nanoparticles.

***Desmostachya bipinnata Linnis* (Kusha)**

Most of the population has used medicinal plants throughout the world for their primary health care needs. There has been exponential growth in the field of herbal medicine and traditional medicines due to the development of advanced technology in chemical analysis and biological activity assessment

nowadays [1].

Fig. 6. *Desmostachya bipinnata* grass.

Desmostachya bipinnata Linnis a perennial grass that is distributed from North Africa to SouthAsia and belongs to the family *Poaceae* or *Graminae*. It is regarded as sacred grass and is used in religious rites [2, 3]. It is a rhizomatous perennial with 2-3 mm thick rhizomes and coarse,narrow, and tough leaves [4]. *D. bipinnata* is the source of vitamins, fiber, minerals, and nutrients for the therapy of different diseases [5]. This review paper has highlighted the phytochemical screening and antimicrobial activities of *D. bipinnata*.

Medicinal Uses

The pharmacological studies revealed that the plant possessed anti-inflammatory, anti- helicobacter, antiulcerogenic, antioxidant, anticancer antimicrobial, anti-diarrhoeal [6], and antiurolithic activity [7]. Roots are used as a cooling, diuretic, galactagogue, and astringent agent. It is also used for urinary calculi, other diseases of the bladder [8], piles, dysuria, carbuncle, cholera [9], and rheumatism [10]. The whole plant is utilized to treat the fistula-in-and [11]. It is also used for the treatment of wounds, abdominal pain, skin diseases [12], calculus, and epistaxis [13]. This plant is also served as a potential remedy to treat several

ailments associated with free radicals [14]. Every part of this plant contributes to its medicinaluse, as summarized in Table 1.

Table 1: Medicinal uses of different parts of *Desmostachya bipinnata.*

S.N	Parts used	Disease cured	Reference
1	Leaf	Wounds, boils, skin	[1]
2	Root	Toothache, stomach disorder, indigestion, dental carrier diuretic, asthma, jaundice cholera, piles, carbuncle	[15] [16] [17] [9]
3	Rhizome	Diuretic, dysentery	[16,18]
4	Whole plant	Airway and gut disorder fistula-in-ano	[19] [11]

Phytochemical Constituents

The phytochemical assessment has resulted in the isolation and identification of various compounds from different morphological parts of *D. bipinnata*. The plant contains vitamins,minerals, carbohydrates, alkaloids, steroids, glycoside, saponins, tannins, phenols, flavonoids, coumarins, volatile oils, lignin, starch, oil globules, and mucilage [20].

Gas chromatography-mass spectrophotometry (GC-MS) analysis of a methanolic extract of leaves of *D. bipinnata* revealed the presence of 10 compounds. The major compound found were benzofuran 2,3- dihydro and *octasiloxane* 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,- hexamethyl with the retention factor 8.9 and 19.7 and peak area 56 and 72, respectively as the major compound [5]. Methanolic extract of leaves of *D. bipinnata* revealed the presence of bioactive compound β-sitosterol-D-glucopyranoside [3]. Five flavonoid glycosides wererereported in 2008 from ethanol extract of aerial parts of *D. bipinnata*, which were identified as kaempferol, quercetin, quercetin-3-glucoside, trypsin (Figure 1), and trypsin-7-glucoside. Out of these compounds, trypsin and trypsin-7-glucoside revealed antiulcerogenic activity [21].

In 2011, five sterols from the leafy culms of *D. bipinnata* were reported and identified as stigmasterol, β-sitosterol, daucosterol, stigmatist-5-en-3β,7β-diol, and stigmatist-5-en-3β,7α-diol [22]. A new xanthene was isolated from methanolic extract, which was identified as 2,6-dihydroxy-7-methoxy-3H-xanthan-3-one (Figure 1) [23]. In 2009, flavonoid compound 4'- methoxy quercetin-7-O-glucoside (Figure 1) was isolated from methanolic extract [24].

GC-MS analysis of the plant essential oil contains β-eudesmol (11.2%), eseroline (25.1%), calarene (3.5%), camphene (16.8%), caryophyllene dioxide (12.3%), isobornyl acetate (9.9%), tricyclene (4.3%), and trans-2,6-gamma-Iron (2.2%). The oil also contained a smaller percentage of endoborneol, (-) caryophyllene oxide, diphenyl iodonium bromide, L-limonene, 2- cyclohexene-1-one, caryophyllene oxide, and 8- nitro-12-tridecanolide [25]. GC-MS chromatogram analysis of an alcoholic extract of the rootstock of *D. bipinnata* results in the isolation of lipid compounds such as *ρ*-hydroxycinnamic acid ethyl ester (16.2%), palmitic acid (15.1%), palmitic acid ethyl ester (9.2%), linoleic acid (6.6%), oleic acid (6.5%), linoleicacid ethyl ester (7.5%), oleic acid ethyl ester (4.5%), stearic acid ethyl ester (2.2%) and 2- methoxy-4-formylphenol [26]. 5-Hydroxymethyl 2-furfural, β-amyrin, β-sitosterol, β- sitosterol-glucoside, stigmasterol-glucoside, and sucrose were isolated from the ethanolic extract of the rootstock of *D. bipinnata* [27]. All these isolated compounds possess excellent phytochemical activity, as summarized in Table 2.

Table 2: Pharmacological activity of compounds isolated from *D. bipinnata.*

S.N	Compounds	Activity	Reference
1.	4′-Methoxy quercetin-7-O-glucoside	Anti-helicobacter	[24]
2.	2,6-Dihydroxy-7-methoxy-3*H*-xanthen-3-en	Anti-cancer	[22]
3.	Trycin and trycin-7-glucoside	Anti-ulcerogenic	[21]
4.	*β*-Sitosterol-D-glucopyranoside	Anti-bacterial	[3]
5.	Camphene	Anti-oxidant	[25]
6.	L-Limonene	Antitumor, Antimutagenic,Anti-inflammatory	[25]
7.	*β*-Eudesmol	Antimutagenic, Antitumor, Antisalmonella	[25]

Antimicrobial Activity

The methanolic extract and essential oils of the plant showed inhibitory action against different organisms. The essential oil extracted by water distillation of the aerial part of the plant possessed a good growth inhibition against Staphylococcus epidermidis (Table 3) [25]. The methanolic extract of the leaf exhibited antimicrobial activity by inhibiting Salmonella typhimurium ATCC 14028 and S. aureus ATCC 259233 [5].

Table 3: Antimicrobial activities of *D. bipinnata.*

S.N.	Extracts	Part of plant	Inhibited micro-organisms	References
1	Essential oil	Aerial part	*S. aureus, S. epidermidis, E. coli, p. auerigonsa*	[25]
2	Ethanol	Whole plant	*M. luteus, B. subtilis, P. mirabilis, S. typhi, S. ventricull, S. aureus, C. tropicalis, C. albicans, A. flavus, A. fumigates, P. chrysogenum, P. aeruginosa*	[29],[32]
		Root stock	*K. pneumonia* (NCIM 2957), *E. coli* (NCIM 2931), *P. vulgaris* (NCIM 2857), *S. typhimurium* (NCIM 2501), *B. cereus* (NCIM 2458 and *E. aerogens* (NCIM 5139)	[31]
		Root	*E. coli, K. pneumonia, S. aureus*	[30]
3	Methanol extract	Leaves	*S. typhi, S. aureus*	[5]
		Aerial	*H. pylori*	[28]
		Whole plant	*H. pylori*	[24]
4	Hydroalcoholic extract	Leaves	*E. faecalis,S. dysenteriae* (ATCC 23513), *P. aeruginosa* (MTCC 741,1688), *S. aureus* (MTCC 3160), *S. aureus* strain (MTCC 96), *P. mirabilis* (MTCC 425) and *K. pneumonia* (MTCC 432)	[3]
5	Petroleum ether	Whole plant	*P. aeruginosa*	[32]
6	Acetone	Whole plant	*P. aeruginosa*	[32]
7	Chloroform	Whole plant	*P. aeruginosa*	[32]

Objectives

Objectives

- One-pot synthesis of Gold Nanoparticles using Desmostachya bipinnata methanolic leaf extract.
- Characterization of synthesized AuNPs using UV-Vis spectroscopy, DLS, Zeta potential, and TEM.
- Comparative antibacterial potential analysis of synthesized AuNPs, Desmostachya bipinnata methanolic leaf extract, and Levofloxacin against E. coli, S. ebony, B. subtilis, and S. aureus.

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).

Methods

Plant collection and Preparation of *Desmostachya bipinnata* leaf extract

Fresh leaves of *Desmostachya bipinnata* were obtained from kursi road Lucknow. The leaf was cleaned with running water, afterwards, they were again washed with double distilled water. Post washing, they were dried at room temperature. 25 grams of leaf were coarsely crushed by mortar-pestle to provide greater surface area.

Afterward extraction process was performed via the Soxhlet method 200ml of methanol was added to the round bottom flask which is attached to a soxhlet extractor and condenser on a heating mantle. The grounded leaves were then tied by muslin cloth and loaded into the thimble, which is placed inside the soxhlet extractor. During the process the temp. was set to 67.4^0C. during the process, the solvent is heated and will begin to evaporate, moving through the apparatus to the condenser and then dripping into the reservoir.

After 10 hr the methanol solvents become yellowish which indicates that the extraction process is completed. Finally, the extract was transferred in a 50ml falcon tube and stored in the refrigerator at 40℃ for further use.

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 *Desmostachya bipinnata* leaf methanolic 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

Results

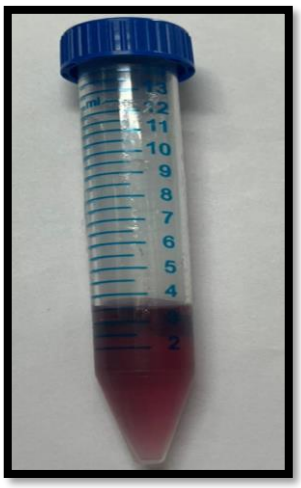

Fig. 7. Synthesized Gold Nanoparticles.

Characterization of Gold Nanoparticles

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.

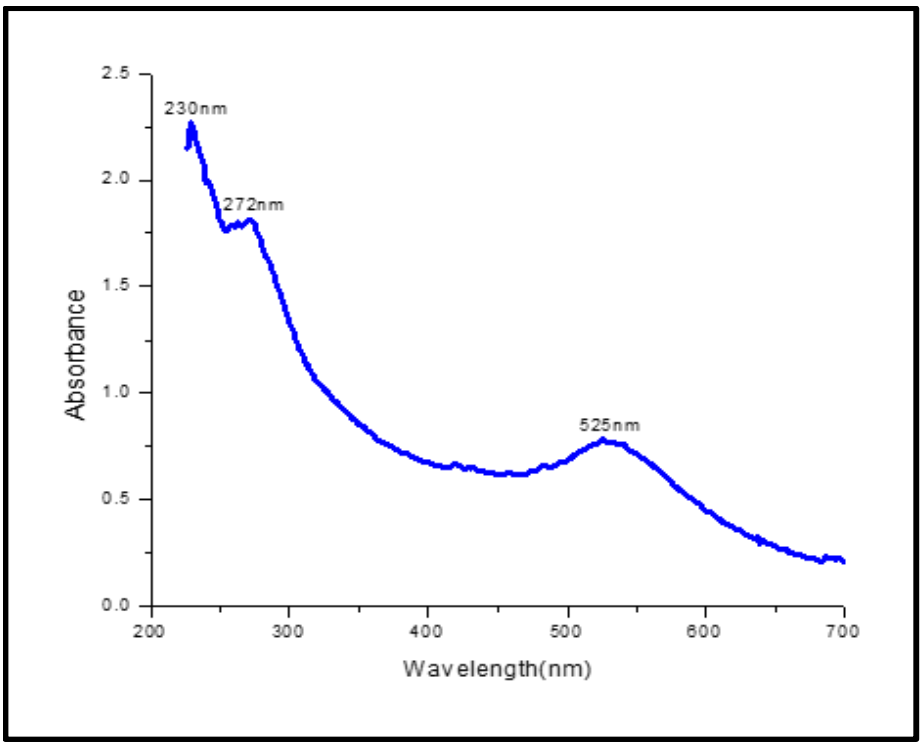

Fig. 8. UV-visible spectroscopy shows the absorption peak of AuNPs at 525 nm.

UV-vis spectra were used to demonstrate the production of AuNPs (figure 1). The absorption peak was found at 525 nm, which coincides with the AuNPs SPR band. *Desmostachya bipinnata* leaf extract phytoconstituents converted the gold salt ($HAuCL_4$) to AuNPs and encapsulated the AuNPs, avoiding the nanoparticles from aggregating and giving stability to the AuNPs. The coloration from light yellow to ruby red indicates that AuNPs had been successfully synthesized and the SPR band's outcome stated this at 525 nm.

Desmostachya bipinnata methanolic leaf extract on the other hand showed no obvious peak. The transmission electron microscope (TEM) was utilized to identify the precise size, shape, and 2-D morphology of AuNPs, which were found to be 17d nm in diameter, spherical in form, and monodispersed in parameter (figure 2).

Furthermore, the average particle size and profile of particle size distribution of AuNPs were determined using the dynamic light scattering (DLS) method. As the seen image (figure 3), AuNPs had an average particle size of 65 d.nm and polydispersity index (PDI) of 0.214, showing a homogenous size distribution. Synthesized AuNP's zeta potentials were also examined (figure 4). In most situations, nanoparticle colloidal stability necessitates a zeta value of -20mV. The synthesized AuNPs have zeta potentials of -17mV, indicating high particle strength. At room temp. no clumping or building of AuNPs was detected in their aqueous dispersion. This was most likely owing to the electronic repellent properties of gold nanoparticles. Because of this repulsion, the nanoparticles are unable to collide.

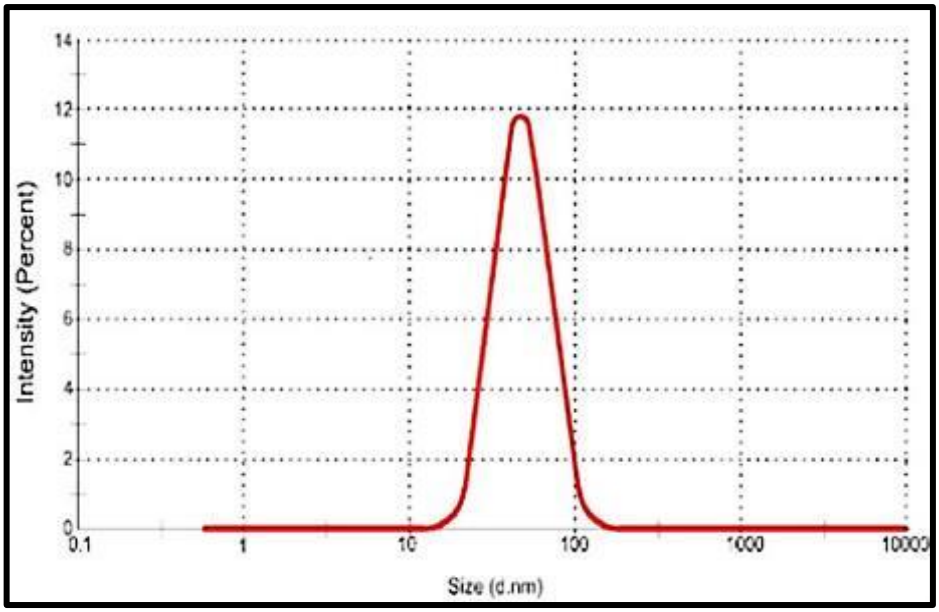

Fig. 10. Dynamic Light Scattering spectra shows the average value of AuNPs (65 d.nm)..

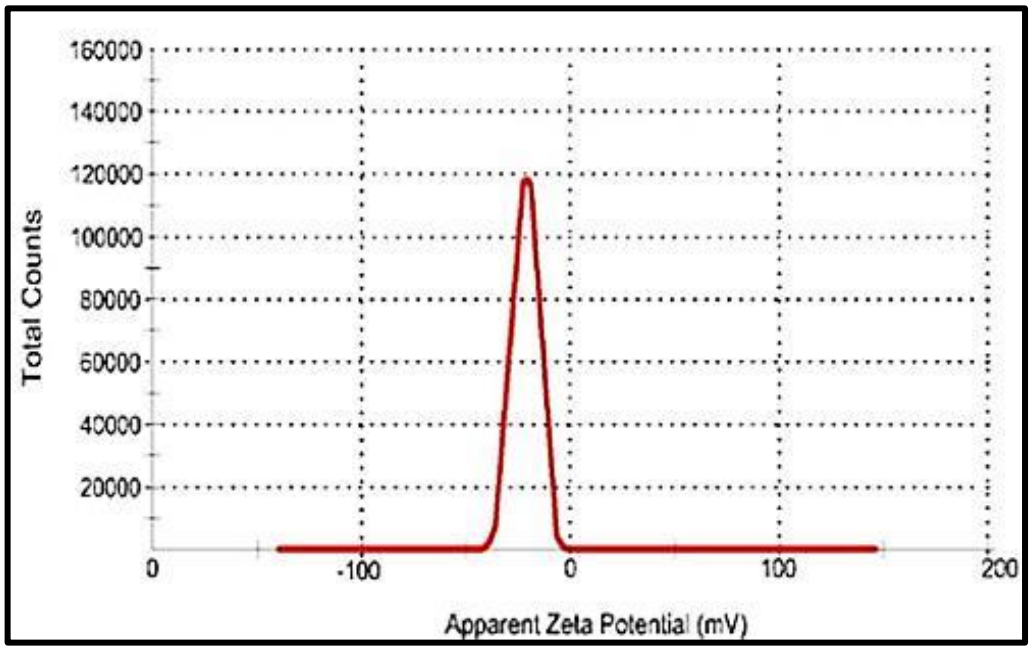

Fig. 11. Zeta potential spectra shows a peak at -17mV.

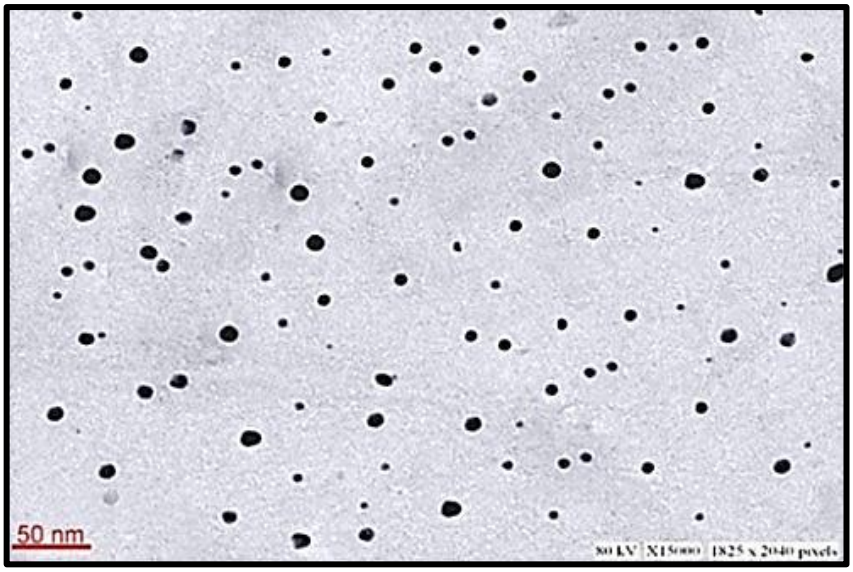

Fig. 9. Transmission Electron Microscope (TEM) micrograph illustrates the size of AuNPs (16 d.nm).

Antibacterial screening

The antibacterial action of synthesized AuNPs against both gram-positive and gram-negative bacterial strains was found to be satisfactory. Using the agar well diffusion method, the antibacterial potential of synthesized AuNPs was evaluated against normal strains of *Escherichia coli, M.luteus, Staphylococcus aureus, and K. pneumonia*. The antibacterial potential was confirmed by a clear zone of inhibition surrounding the inoculated region. The maximum zone of inhibition was found against Escherichia coli.

S. No.	Bacterial Species	Gold Nanoparticle (Zone of Inhibition in mm)		
		AuNPs (50µg/well)	Antibiotic	Plant extract
1.	*Escherichia coli*	16	24	7
2.	*M. luteus*	15	26	8
3.	*Staphylococcus aureus*	11	20	6
4.	*K. pneumoniae*	10	18	5

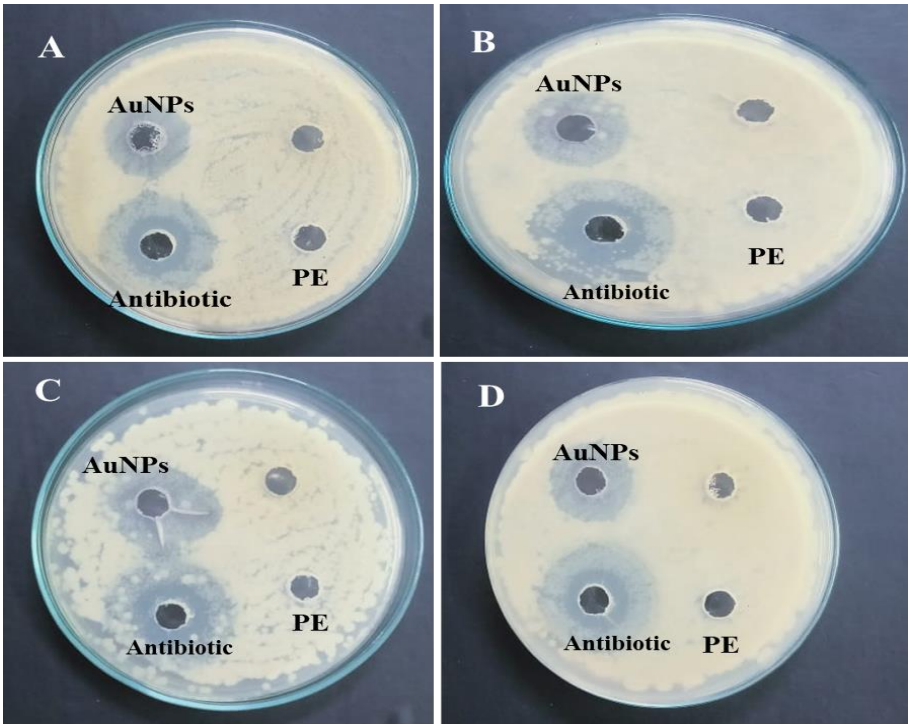

Fig. 12. Synthesized AuNPs shows Antibacterial Activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia*, and *M. luteus* (Well diffusion, method).

Zone of inhibition

The antibacterial studies with 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 in *S. aureus***,** *M. luteus, E. coli, and K. pneumoniae* gram-positive and negative bacteria.

MIC (Minimum Inhibitory Concentration)

We found that biogenic AuNPs synthesized by *Desmostachya bipinnata* methanolic leaf extract show strong antibacterial activity against both Gram-positive and Gram-negative pathogenic bacterial strains. The MIC50 of 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 AgNPs 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.

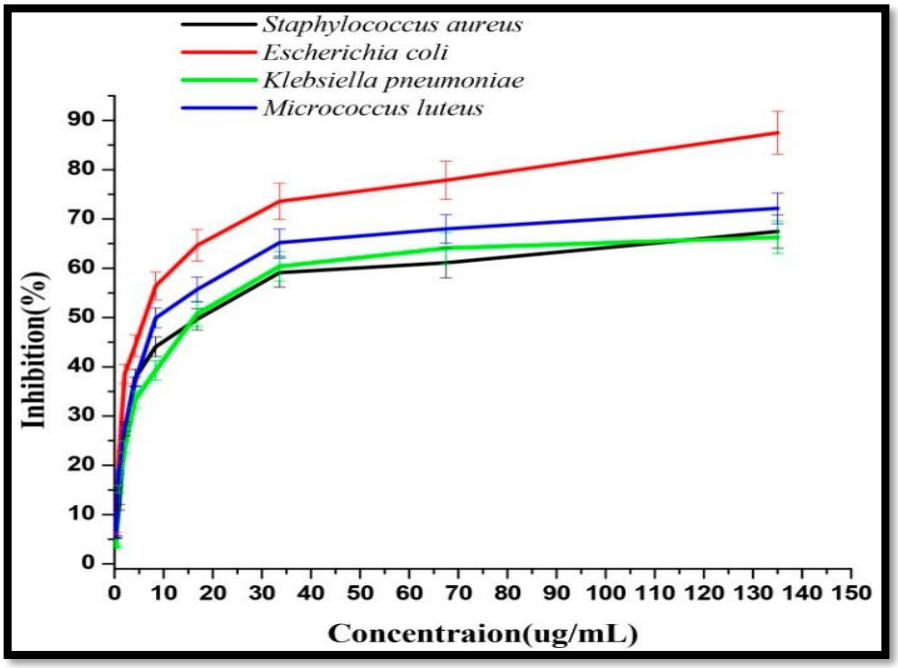

Fig. 13. Antibacterial potential of AuNPs (8µg/ml) against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *M. luteus* (96 well plate method).

Discussion and conclusion

Discussion

Desmostachya bipinnata methanolic leaf extract was utilized as a reducing and stabilizing agent in this work. The *Desmostachya bipinnata* methanolic leaf extract reduces enzymes and capping agents, such as secondary metabolites. 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 UV the 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, *Desmostachya bipinnata* methanolic leaf extract was used for the synthesis of 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 529nm, due to the surface plasmon resonance property of Gold Nanoparticles.

Therapeutic Analysis was performed for the AuNPs for antibacterial assay. A synthesized gold nanoparticle from *Desmostachya bipinnata* methanolic leaf 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 *Desmostachya bipinnata* methanolic leaf extract. As a previous study, the plant has anticancer, antioxidant, antidiabetic, and antibacterial, activity.

These biogenic AuNPs exhibited significant dose-dependent antibacterial potentials. However, further investigations are warranted to assess the toxicity details and the mechanism associated with the antibacterial and anticancer action of the biosynthesized AuNPs. Nevertheless, the outcomes of the present study provide a broad AuNPs-based platform for various therapeutic applications soon.

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