A DISSERTATION ON

"Green synthesis of gold nanoparticles by *Parthenium hysterophorus* leaf extract and investigation of their cytotoxic potential against cervical cancer HeLa cells"

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTERS OF SCIENCE
IN BIOTECHNOLOGY

BY

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M.Sc. BIOTECHNOLOGY (IVth SEMESTER)

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. SAUMYA** student of M.Sc. Biotechnology, (IV semester), Integral University has completed her four months dissertation work entitled "**Green synthesis of gold nanoparticles by Parthenium hysterophorus leaf extract and investigation of their cytotoxic potential against cervical cancer HeLa cells"** successfully. She has completed this work from the Department of Biosciences, Integral University, under the supervision of **DR. IRFAN AHMAD ANSARI**. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

Dr. Sanober S.Mir

Head Of Department

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. SAUMYA** during the months February – June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the script of thethesis has been written by the candidate herself. The thesis entitled is ***"Green synthesis of gold nanoparticles by Parthenium hysterophorus leaf extract and investigation of their cytotoxic potential against cervical cancer HeLa cells" "*** therefore, being forwarded for the acceptance in partialfulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow.

SUPERVISOR

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SAUMYA

M.Sc BIOTECHNOLOGY

LIST OF FIGURES, GRAPHS AND TABLE

ABBREVIATIONS

%	Percentage
M	Molar solution
mM	milli Molar
CNTs	Carbon nano tubes
QDs	Quantum dots
DLS	Dynamic Light scattering
OD	Optical density
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
UV-Vis	Ultraviolet visible spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-ray Diffraction
Nm	Nano meter
MIC	Minimum Inhibitory Concentration
AgNPs	Silver nanoparticles
NPs	Nanoparticles
PARTH	Parthenium hysterophorus

INTRODUCTION

Introduction

Nanoscience is the study of structures and molecules on the scales of nanometers ranging between 1 and 100 nm, and the technology that utilizes it in practical applications such as devices etc. is called nanotechnology *(*Mansoori*. et al.,* 2005*).* Nanotechnology is concerned to use structures by controlling their shape and size at nanometer scale. The second issue has to do with novelty: nanotechnology must deal with small things in a way that takes advantage of some properties because of the nanoscale *(*Allhoff*. et al.,* 2009*).* The American physicist and Nobel Prize laureate Richard Feynman introduce the concept of nanotechnology in 1959*(*Feyman *et al .,*2018). After the discovery of feyman two approaches have been developed top-down and bottom up which differ in speed, quality and coast. top-down is breaking of bulk material in nano-sized particles and bottom-up approach refers to the build-up of nanostructures from the bottom by physical and chemical methods which are in a nanoscale range (1 nm to 100 nm) using controlled manipulation of self-assembly of atoms and molecules *(*Ibal*. et al.,*2012*).* The novel biological, chemical, or physical properties of nano material are mainly because of their size (usually less than 100 nm) that increases their surface area to volume ratios drastically *(*Zhang. *et al* 2020). Using nanotechnology, it may be possible to achieve (1) improved delivery of poorly water-soluble drugs; (2) targeted delivery of drugs in a cell- or tissue-specific manner; (3) transcytosis of drugs across tight epithelial and endothelial barriers; (4) delivery of large macromolecule drugs to intracellular sites of action; (5) co-delivery of two or more drugs or therapeutic modality for combination therapy; (6) visualization of sites of drug delivery by combining therapeutic agents with imaging modalities and (7) real-time read on the in vivo efficacy of a therapeutic agent . Although at an earlier stage, applications of nanotechnology for prevention and treatment of HIV/AIDS have also gained attention in recent years *(*Ferrari*.et al.,* 2005).

Nanoparticles (NPs) are defined as particles with one dimension ranging between 1 and 100 nm. NPs exhibit different properties depending on their size and surface functionalities (Gwinn.*et al.,* 2006). The small size and large surface area account for the extensive use of NPs in various areas such as cosmetics, electronics and both diagnostic and therapeutic medical applications (Missaoui.et al .,*2018)* .The exponential growth and increasing interest in nanotechnology have been enhanced by

the ability to image nanomaterials using techniques with atomic resolution capabilities such as scanning tunneling microscopy, scanning transmission electron microscopy and tandem electron microscopy *(*Sharma, *et al .,* 2019). The exponential growth of nanotechnology has led to studies focusing on the associated risks of NPs and nanotechnology in general. However, and despite our increased exposure to NPs, information regarding NP safety is lagging behind as compared to the research on the application of. NPs are used as pharmaceutical drug carriers with applications in both diagnostics and therapy. These NPs, including polymeric NPs, nanoemulsions, liposomes and solid NPs, are suggested to have potential clinical applications. Their clinical applicability depends on different parameters such as their physical and chemical properties, drug loading efficiency, drug release and most importantly low or no toxicity of the carrier itself *(*Puri.*et al.,*2009).A recent advancement in nanotechnology is based on the synthesis of NPs/materials using novel techniques. NPs can be fabricated using physical, chemical and biological methods.The physical method involves pyrolysis, crushing, grinding, ball processing. On the other hand, chemosynthesis of nanoparticles involves. Chemicalreduction, electrolysis, CVD (Chemical vapor deposition),photocatalytic reduction. However physical method produces less yield of NPs and high coast and energy involvement (Shedbalkar.*et al.,* 2014). Chemical method is unsafe to use as they are toxic, hazardous, carcinogenic, potent environmental pollutant *(*Arshad.*et al.,* 2017*).* Eco friendly alternatives to Chemical and physical methods are biological ways of nanoparticles synthesis using microorganisms, enzymes, fungus, and plants or plant extracts *(*Ahmad.*et al.,* 2011*).* NPs have been shown to induce oxidative stress in the liver, spleen and kidneys and inhibit the effects of antioxidants *(C*outo*.et al.*, 2016*).* NPs are also used as gene delivery systems and have shown efficacy in replacing specific target diseased genes involved in cancer (Kaul.*et al., 2*005). NPs' size plays an important role in their entry route, cellular uptake and overall toxicity. Some studies suggest a direct correlation between the size of NPs and their distribution and the level of ROS generation in the kidneys *(Shar*ifi.*et al.,* 2012*)*

Gold nanoparticles are of interest for various applications in diagnostics and therapeutics and several reviews discuss the unique properties and utilities of AuNPs *(*Alkilany.*et al.,* 2010*).* AuNPs can scatter light intensely and are photo stable, making particles attractive tools for imaging *(*Huang.*et al., 20*07*)* As reviewed by Han et al.,

AuNPs can also be used as drug or gene delivery vehicles because they can be easily functionalized with ligands such as targeting agents and cell penetrating peptides *(*Han. *et al.,* 2007*)* general AuNPs allow site-specific delivery of therapeutics, thus reducing systemic side effects and enhancing treatment efficacy *(*zolnik.*et al.,* 2009*).* A major application of AuNPs could be in the field of medicine as an antimicrobial agent. AuNPs have been reported to be non-toxic compared to other metallic NPs*. (*Asharani.*et al., 2*011*).* Other applications include control of pollution, purification of water and hydrogen, and as catalysts in oxidation of carbon monoxide *(*Mcpherson.*et al.,* 2009*)*

Microbial synthesis of AuNPs: AuNPs can be generated by using microorganisms due to the need for environmental-friendly and low cost procedures. Biological synthesis of nanoparticles has been at the center of attention as a green and eco-friendly method in current years. In biological methods, nanoparticles are synthesized by microorganisms, enzymes, and plants or plant extracts *(*Mohanpuria *et al.* 2008*).* Gold nanoparticles of different sizes, ranging from 1 nm to 8 m and shapes including spherical, octahedral, sub-octahedral, decahedral multiple twinned, icosahedral multiple twinned, irregular shape, nanotriangles and nanoprisms, tetra hedral, hexagonal platelets and nanorods

Recently, the use of plants for the synthesis of nanoparticles is gaining importance, because of their availability, low cost, eco-friendliness and non-toxic nature. The extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way. The nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics summarizes some of the reports pertaining to nanoparticle synthesis 210 mediated by extracts of various plants. *(*Dwivedi. *et al.,* 2010*). The* heterogeneity of the size and morphology of nanoparticles produced in whole plants may hinder their use in applications where specific, finely tuned sizes and shapes are requiredin vitro approaches have actively been developed in recent years which provide a more flexible control over the size and shape of the nanoparticles (for example, by changing the medium pH and reaction temperature), as well as facilitating easy purification (Rai M Yadav. et al., 2013*). Application*of nanoparticles synthesized in plant extract are that

traditional physiochemically synthesized metal nanoparticles have been used in cancer therapy, the targeted delivery of drugs, molecular imaging, wastewater treatment, catalysis, biosensor development, fuel elements, coatings, cosmetics and as antiseptics. *(*Dhanalakshmi*.et al.,* 2012*)*

REVIEW OF LITERATURE

Review of Literature

Nanotechnology

Nanotechnology is an emerging branch of science which deals to with excising the structure of matter between 1 and 100 nanometers on the atomic, molecular and super molecular level for the development of desired properties and functions and for diverse applications (Mansoori *et al.,*2005). The American physicist and Nobel Prize Laureate Richard Feynman introduce the concept of nanotechnology in 1959. (Lipinski *et al.,* 2011). A Japanese scientist was the first to use and define the term "nanotechnology" in 1974 as: "nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule (Zackcey *et al.,* 2014). Nanotechnology has advanced exponentially in the last decade and many products containing nanoparticles are now used in various applications such as in food science, cosmetics and pharmaceuticals. Today, it is estimated that there are more than 1,600 nanotechnology-based consumer products, with new ones entering the market at a rapid pace (Yang yu *et al.,* 2014).One of the key applications of nanotechnology is in medicine, especially for the treatment of cancer (Dayakar *et al.,* 2017). Engineering matter on a nanoscale can give nanoparticles (NPs; nanomaterials that are < 100 nm in size) unique properties that small molecules and bulk materials do not possess. These properties have been used for the development of novel medical diagnostics and therapeutics. Nanotechnology has made a strong impact on medical imaging and diagnostics. In imaging, advances in nanotechnology have resulted in the clinical translation of iron oxide NPs as MRI contrast agents. The most extensively studied agent is the lymphotrophic super paramagnetic iron oxide NP ferumoxtran-10, which can be used to detect subcentimeter lymph node metastases. Nanotechnology has also been applied in x-ray generation. Carbon nanotubes (CNTs) have been used as electron emitters in x-ray imaging (Wu Chan *et al.,* 2013). Because of its ability to generate x-ray beams on the micrometer scale, CNT has also been used in the development of a microbeam radiation therapy system. Nanotechnology has also been incorporated into devices to capture circulating tumor cells (CTCs). CTC devices also allow early detection of cancer and real-time monitoring of disease. The significance of CTCs is being explored for use in radiation oncology (Li Yang *et al.,* 2012) .One of the most hotly researched subfields of nanotechnology is nanomedicine, which increases the possibility of specific targeted cancer therapy (Liu *et al .,* 2016). Moreover, nanotechnology is also a useful tool for cancer

detection, and monitoring the disease as it metastasizes. To date, nanotechnology has been applied in the detection and diagnosis of various cancers, such as cervical cancer, lung cancer, breast cancer, gastric cancer, nasopharyngeal cancer, and oral cancer (Liu. *et al* 2016). Although at an earlier stage, applications of nanotechnology for prevention and treatment of HIV/AIDS have also gained attention in recent years. There are emerging novel approaches in which nanotechnology can enhance current treatment as well as advance new therapeutic strategies, such as gene therapy and immunotherapy. Moreover, some nanomaterials have therapeutic effects by themselves. Nanotechnology can also play a major role in preventive strategies for developing vaccines and microbicides. The use of nanotechnology systems for delivery of antiretroviral drugs has been extensively reviewed by (Nowacek *et al.,* 2016).The most clinically advanced application of nanotechnology for immunotherapy of HIV/AIDS is the DermaVir patch that has reached Phase II clinical trials. Additional nanotechnological-based detection and therapeutic devices were made possible using photolithography and nucleic acid chemistry (Cao *et al.,* 2009). Full potential of nanotechnology has yet to be realized in most industry sectors, medicine has benefited and been influenced by this field for several years, particularly in oncology. The premier drug-delivery nanoparticle currently in clinical use is the liposome nanotechnology offers novel and improved solutions for the localized release of the biomolecules and growth factors that are needed in any TE approach. Nanotechnology in TE overcomes many downfalls that micron structured implants face, such as infection, chronic inflammation, and poor binding with the surrounding tissue. To improve these issues, nano-scale features have been implemented, providing enhanced biointegration (Hadsell *et al*., 2013*)* The nanotechnology revolution has had a ground-breaking impact in various fields such as chemistry, biology, and engineering (Martin *et al .,* 2014) The number of nanotechnology application is rising in different fields such as biomedicine, robotics, electronics, automobiles, and civil engineering industry (including transportation) because of their superior performance (Gharat *et al .,*2016). This key enabling technology has evidenced broad and remarkable applications in diverse fields such as electronics, medicines, cosmetics, textiles, food science, energy sector and agriculture.The encouraging development of nanotechnological approaches in agriculture particularly for crop productivity and disease management is shown by current trends of publications and patents several studies have addressed how nanotechnological approach is benefiting the agricultural sector in number of ways. For examples: pesticides encapsulation in nanoparticles for their sustained release; nanoparticles mediated delivery of genetic

material for crop improvement; carbon nanotube assisted seed germination of rain-fed crops; nanofertilizer for enhanced crop nutrition & crop productivity; nanopesticide for plant disease management; nanoherbicide for weed elimination and nanosensors for detection and forecast of pathogens and soil monitoring. The growing challenge of global food security and climate change strongly underline the commercial applications of nanotechnology-based products for agricultural sector (Rossi *et al.,* 2014). Nanotechnology has enabled the production of packaging that is active and intelligent with improved mechanical and thermal properties to ensure better protection of foods (Han *et al.,* 2018). Nano-enabled solutions also allowed the integration of various bioactive molecules and nanoparticles to prevent oxidation and food degradation. Selenium and cellulose NPs can be integrated into food packaging to retard or inhibit the ROS that can degrade food quality (Halo *et al.,* 2014). The use of nanostructures for removing microorganisms or toxins from water has been common (Zdobnova et al., 2011*).*RNA nanotechnology focuses on utilizing the properties of RNA to build architectures with nanomedicine applications. Classical studies on RNA structure and function focuses on intra-RNA interactions and 2D/3D structure–function relationships, whereas RNA nanotechnology focuses on inter-RNA interactions and quaternary interactions of RNA motifs. However, the fields of RNA research are not exclusive in their information. Much of the pre-existing RNA biology research is utilized extensively in RNA nanotechnology. For example, many RNA nanoparticles use functional RNAs such as ribozymes, riboswitches, and miRNAs (Fabin*. et al.,*2014).

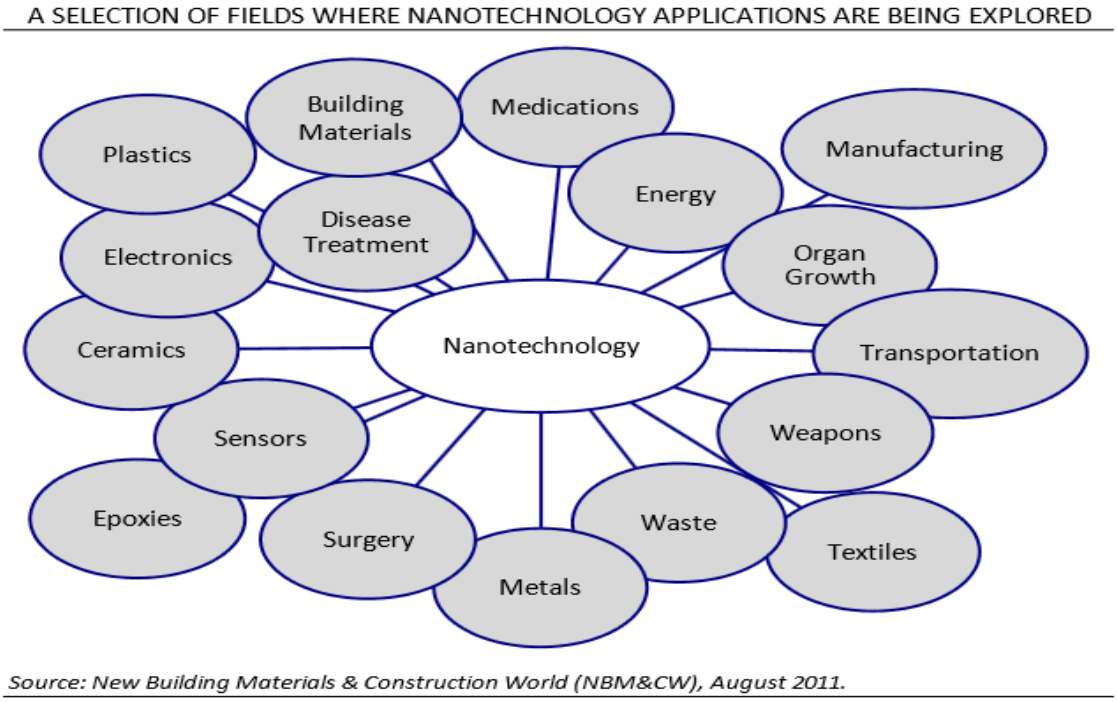


Figure1: A selection of fields where nanotechnology applications are being exposed

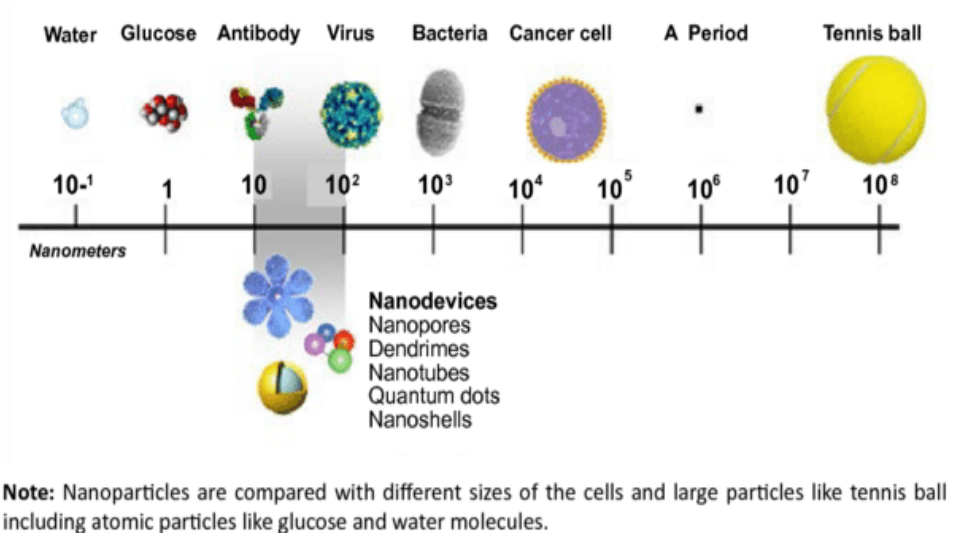
Nanoparticles

Nanoparticle generally refers to a particle with a size ranging from 1 to 100 nm. (Kunjachan *et al.,*2012).Nanoparticles can be broadly classified into two major classes such as organic materials, which are liposomes, dendrimers, carbon nanotubes, emulsions, and other polymers, and inorganic materials, which include metals. Nanoparticles can be synthesized in different sizes (1.0–500 nM) and shapes (cones, cubes, rods, tubes, and shells).(Kircher *et al 2*012). The majority of these nanoparticles are used for therapeutic purposes (Kaittanis *et al,* 2014). Currently, several therapeutic nanoparticles are applied in clinical practice. Doxil (PEGylated, doxorubicin-loaded liposomes, Janssen, Horsham, PA), Abraxane (paclitaxel-containing albumin nanoparticles, Celgene, Summit, NJ), and AmBisome (liposomal amphotericin B, Gilead, Foster City, CA) are some prominent examples of clinically approved therapeutic nanoparticles; many other nanomedicine formulations are currently being tested in preclinical and clinical trials (*Cai* et *al.,*2011). Numerous nanoparticle-based imaging agents have been proposed for diverse imaging modalities including CT, MRI, SPECT, US, OI, and PAI (*Seve et al.,* 2012). The most important properties of

nanoparticle formulations comprise particle size and charge, core and surface properties, shape and flexibility, as well as multivalency and controlled synthesis, as they determine the in vivo distribution, targeting ability, and toxicity of the nanoparticle. Furthermore, these properties have a strong impact on drug loading capacity and release, and on the stability of nanoparticles (*A*dars*h* et *al.,* 2017). Nanoparticles also offer the potential to protect the encapsulated molecules from both extracellular and intracellular degradation, resulting in improved intracellular bioavailability.The surfaceof nanoparticles is commonly coated with a layer of dissolved extracellular molecules in body fluids, such as proteins, sugars and lipids before their encounter with the cellular membranes, the so-called protein corona(Jackson.*et al.,* 2016). Between 1970 and 2015, 359 applications for drug products containing nanomaterials were submitted to the US Food and Drug Administration (FDA)'s Center for Drug Evaluation and Research (CDER).The applications of nanoparticles in diagnosis and therapy have been increasing over the years. Due to their size (1–100 nm), nanoparticles have a large surface area-to-volume ratio, which allows them to absorb high quantities of drugs (Nie *et al.,* 2007). and to be spread easily throughout the bloodstream. Nanoparticles (NPs) are the main system used in nanomedicine, as theranosticagents with high molecular specificity (Jaque *et al.,* 2014) nanoparticles are of great interest, because of their antioxidant properties and easy internalization by the cells (Yong *et al.,* 2013*)*

Types of nanoparticles

NPs can be divided into three main groups: organic nanoparticles (liposomes and polymers), inorganic nanoparticles (metals, metal oxide, ceramic, and quantum dots), and carbon-based nanoparticles (Lori e*t al.,* 2007*)*



Note: Nanoparticles are compared with different sizes of the cells and large particles like tennis ball including atomic particles like glucose and water molecules.

Figure2: Nanoparticles with different sizes

Quantum dots (QD)

Quantum dots (QDs) are semiconductor nanosized crystals that have been intensively used for fluorescence bioimaging (Caruthers *et al.,* 2007). In general, quantum dots (QDs) consist of a semiconductor core (e.g., cadmium–selenium (CdSe), cadmium–tellurium (CdTe), indium–phosphate (INP), or indium–arsenate (InAs)), overcoated with a shell (e.g., zinc sulfide (ZnS)) to improve their optical and physical properties and to prevent leaking of the toxic-heavy metals (Nam *et al., 2004*). These nanoparticles are the most used in bioimaging and biosensing strategies. When an appropriate surface treatment is performed, QDs have negligible toxicity (Zang *et al*., 2008). QDs' biocompatibility is essential for their biological and biomedical applications. Biocompatible QDs can be obtained through three different routes: (1) biomimetic synthesis, through either the use of artificial cellular structures or biomolecules (nucleic acids, peptides, proteins, and enzymes) as templates; (2) biosynthesis, using living organisms in bioreactors; or (3) modifying the surface of QDs derived from chemical synthesis. The biosynthetic approach provides a green pathway for preparing biocompatible QDs without generating toxic products or aggressive reaction conditions, one of the most important QDs are gold quantum dots (GQDs), which have similar properties to those of gold nanoparticles; however, unlike other QDs, they do not display fluorescence. Depending on solvency, shape, particle size and ligand, surface functionalization, dielectric properties, medium, and agglomeration, which render them highly useful in biological system detection applications, such as DNA sequencing,

hybridization assays, genetic disorders, flow cytometry, and immune blotting *(*Dong *et al.,* 2007). In this sense, synthesized GQDs and functionalized them with a peptide moiety containing a nuclear export signal and caspase-3. GQDs function as molecular probes for real-time monitoring of cellular apoptosis, making them ideal for use in cancer.

Carbon based nanoparticles

These include fullerenes and nanotubes. Fullerenes are novel carbon allotropes with a polygonal structure made up exclusively of 60 carbon atoms *(*Meyers *et al.,* 2009). Carbon nanotubes are normally manufactured from chemical vapor deposition of graphite. There are two classes of carbon nanotubes: single-walled (SWCNT) and multiwalled (MWCNT), the latter of which exhibits potent antimicrobial properties (Carbon-based nanoparticles are considered of interest in biomedical applications due to their physical properties, including high electrical conductivity and excellent mechanical strength, but they are not biodegradable. Carbon nanotubes (CNTs) have outstanding optical properties, which is why they are used as labeling and imaging agents (Meyers *et al* 2009) several studies have reported cytotoxicity induced by CNTs. increased cytotoxicity has often been attributed to incomplete removal of metal catalysts used to prepare CNTs (Lellouche *et al.,* 2009) Most in vivo studies using CNTs have shown them to be safe, as toxicity was not reported and there was renal clearance from the body, although small portions of CNTs were found in certain organs, such as the liver, spleen, and lungs *(*Wu *et al.,*2014*)*

Polymeric Nanoparticles

Most polymeric nanoparticles are known for their biodegradability and biocompatibility, constituting the most commonly used NPs in drug delivery systems (Salahuddin N.L *et al* ., 2015)This type of nanoparticle can be made from natural polymers, such as chitosan, or synthetic polymers, such as polylactides (PLA), poly (methyl methacrylate) (PMMA), or polyethylene glycol (PEG) *(*Sirelkhatim *et al.,*2015*)* Polymeric nanoparticles can be prepared by different methods, including two-step procedures based on emulsification, emulsification-solvent evaporation, emulsification-solvent diffusion, and emulsification–reverse salting-out. Additionally, there are methods such as one-step procedures involving nanoprecipitation methods, dialysis and supercritical fluid technology. Among the techniques used to analyse surface properties, we can find energy dispersive spectroscopy (EDS), zeta potential (ζ-potential), X-ray photoelectron

spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), and Raman. These techniques reveal the chemical composition of polymeric nanoparticle surface and surface functionalization .in order to improve drug-loading efficiency and prolong drug release, the nature of polymer-drug interactions, as well as the polymer type and its physicochemical properties, must be considered *(*Yamada *et al .,* 2007)

Metallic Nanoparticles

These include precious metals (gold or silver) and magnetic metals (iron oxide or cobalt and manganese doped ferrites). Metallic nanoparticles such as gold (Au) possess unique electronic and optical properties and are nontoxic and biocompatible, and their surface can be modified with other biomolecules due to their negative *charge (*ZangY *et al.,* 2013). A gold surface offers a fantastic opportunity to conjugate ligands such as proteins, oligonucleotides, and antibodies containing functional groups such as phosphines, thiols, mercaptans, and amines, which have a high affinity for the gold surface. Gold nanoparticles have applications in imaging techniques for the diagnosis of various diseases (Martinez *et al.,* 2018*).* El-Sayed et al. established the use of gold nanoparticles (AuNPs) for cancer imaging by selectively transporting AuNPs into the cancer cell nucleus. In order to do this, they conjugated arginine–aspartic acid–glycine peptide and a nuclear localization signal peptide to a 30 nm AuNPs.

Metal Oxide Nanoparticles

These NPs exhibit catalytic and antioxidant activities, chemical stability, optical properties, and biocompatibility, all of which make them suitable for several biomedical applications. The most widely used are iron oxide (Fe_3O_4), titania (TiO_2), zirconia (ZrO_2), and more recently, ceria (CeO_2). For instance, Titania nanoparticles are incorporated into medical implants due to the biocompatibility of their surface, and ceria nanoparticles are the object of increasing attention because of their catalytic and antioxidant capacity, which allows them to act as antioxidant and anti-inflammatory agents (Felix *et al.,* 2014*).* The biomedical applications of these nanoparticles are increasing due to their unique properties, which include large specific surface area, pore volume, controllable particle size, and good biocompatibility. It is due to these properties that mesoporous silica nanoparticles have been investigated for their use in drug delivery in biomedicine and biosensors. Studies have demonstrated the effectiveness of the use of other nanoparticles, such as zinc oxide (AuNPs), in drug delivery and bioimaging. Important

characteristic of gold nanoparticles is that their surface needs to be modified to protect them in biological systems, as they can be easily dissolved in water and acidic solutions. Furthermore, in order to use AuNPs nanoparticles for fluorescence in imaging, they first need to be doped, as the bandwidth is in the UV region and UV light cannot penetrate tissues and can be harmful to cells and tissue *(*Mosnier *et al.,* 2016)

Ceramic Nanoparticles

These are inorganic compounds with porous characteristics that have recently emerged as vehicles for drugs. They are capable of transporting molecules such as proteins, enzymes, or drugs without swelling or compromising their porosity due to the external effects of pH or temperature *(*Giesa *et al.,* 2011*)* the components most commonly used in ceramic nanoparticles are silica and aluminum. core of these nanoparticles is not limited to these two materials. they can be composed of a combination of metallic and nonmetallic materials. For instance, CeO_2-capped mesoporous silica nanoparticles (MSN.) act as vehicles for drug delivery by releasing β-cyclodextrin into lung cancer cells *(*Panwa. *et al.,*2015*)* There are a wide range of ceramic materials with multiple applications, including clay minerals, cement, and glass. Biocompatible ceramics, also known as bioceramics, are mainly used for the bone, teeth, and other medical applications. The most widely used ceramic nanobiomaterials are calcium phosphate (CaP), calcium sulphate and carbonate, tricalcium phosphate (TCP), hydroxyapatite (HAP), TCP+HAP, bioactive glasses, bioactive glass ceramics, titania-based ceramics, alumina ceramics, zirconia ceramics, and ceramic polymer composites. All have been applied in nanomedicine, orthopedics, bone regeneration, dentistry, and tissue development, in addition to other biomedical uses in the human body *(*Ahsan *et al.,* 2020*)*

Liposome Nanoparticles

These are spherical vesicles with a membrane composed of a lipid bilayer containing an aqueous substance. The amphiphilic molecules used for the preparation of these vesicles are similar to biological membranes so as to improve the efficacy and safety of different drugs. Liposomes can be synthesized by sonicating a dispersion of amphipatic lipids, such as phospholipids, in water. In fact, low shear rates can create multilamellar liposomes. Sonication is a “gross” method of preparation, as it can damage the structure of the drug to be encapsulated. In addition, there are other methods, such as

extrusion and the Mozafarimethod, which are employed to produce materials for human use. It is important to mention that using lipids other than phosphatidylcholine can greatly facilitate liposome preparation. Liposomes are mainly used for delivering chemotherapeutic drugs in cancer treatment (Lou *et al.,* 2012*)*. They can also incorporate a high number of bioactive materials, including pharmaceutical drugs or food ingredients. Liposomes have great potential applications in nanomedicine, as well as in the food and cosmetics industries, due to their high biocompatibility and biodegradability. , nanoliposome technology has become highly developed, offering real opportunities to food technologists in areas such as the controlled release and encapsulation of food ingredients , liposomes are being used as an advanced technology to carry active molecules to specific targets *(*Breaker *et.al.,*2012*)*

Gold Nanoparticles

Gold nanoparticles (AuNPs) have been widely employed in bionanotechnology based on their unique properties and multiple surface functionalities. The ease of AuNP functionalization provides a versatile platform for nanobiological assemblies with oligonucleotidesantibodies and proteins. Bioconjugates of AuNPs have also become promising candidates in the design of novel biomaterials for the investigation of biological systems (Taranova *et al.,* 2017). In diagnostics, the binding event between the analytes and the AuNPs can alter the physicochemical properties of AuNPs such as surface plasmon resonance, conductivity, and redox behavior, leading to detectable signals. AuNPs also serve as practical platforms for therapeutic agents, with their high surface area allowing a dense presentation of multifunctional moieties (e.g., drugsand targeting agent). Developed a synthetic method for creating AuNPs in 1951 by treating hydrogen tetrachloroaurate ($HAuCl_4$) with citric acid in boiling water, where the citrate acts as both reducing and stabilizing agent. Ferns further refined this method by changing the gold-to-citrate ratio to control particle size. This protocol has been widely employed to prepare dilute solutions of moderately stable spherical AuNPs with diameters of 10 to 20 nm, though larger AuNPs (e.g., 100 nm) can also be prepared.

Synthesis of nanoparticles

There are two methods for the synthesis of metallic nanoparticles- top-down and bottom-up.Gold nanoparticles can be synthesized using chemical, electrochemical and biological (green) synthesis techniques.

(i) **Chemical reduction Method:** Chemical reduction method is the most common and widely used method for the preparation of gold nanoparticles. This method includes, in the presence of reducing agent, gold salt is reduced. Synthesis of AuNPs was based on a single-phase reduction of gold tetrachloroauric acid by sodium citrate in an aqueous mediumand produced particles about 20nm. The major contribution for AuNPs synthesis was published in 1994 and now it popularly known as Burst Schifrin method (D'Mello*.et al.,* 2017)*.*The major benefit of synthesizing this process are ease of preparation, size controlled, and thermally stable nanoparticles and reduced dispersity.

(ii) **Electrochemical method:** The electrochemical production of nanoparticles was first studied by Reetz et al., in 1994*(*Stapleton *et al.,* 2014*).* Their studies showed that size- selective nano scale of transition metal particles could be set electrochemically, using tetra alkyl ammonium salts as stabilizers of metal clusters in a non-aqueous medium.

Gold nanoparticles on the surface of multi-walled carbon nanotubes with glassy carbon electrodes can be prepared using the electrochemical synthesis technique.The gold nanoparticles were prepared electrochemically using a simple two-electrode cell, with oxidation of the anode and reduction of the cathode.

(iii) **Biological (green) method:** In recent years, research on the synthesis of nanoparticles using green materials, commonly called green synthesis, has been growing. The proposed materials are types of microorganisms, enzymes, plants, or plant extracts. In the production of biocompatible nanoparticles, the use of plants or microorganisms is increasingly being used, and nanoparticles from such "green synthesis" have been applied for drug and gene delivery and various medical treatments including antimicrobial, anticancer, anti-inflammatory, anti-aging, antioxidant and anti-biofilm inhibition *(*Geetha *et al.,*2013*)*

Most of the conventional methods used to produce nanoparticles have disadvantages such as the use of toxic chemicals and the generation of waste, which can cause environmental pollution. Consequently, in recent years there has been increasing interest in eco-friendly synthesis methods. The methods involve the use of organisms including bacteria, fungi, and plants, which can reduce metal salts and enable the

formation of nanoparticles that present the desired size and morphology. The production of nanoparticles by biological reduction of metals is an option that can be considered clean, non-toxic, and environmentally acceptable.

- **Synthesis of gold NPs by bacterial strains**

The Fe (III)-reducing bacteria like *Geobacter sp. Magnetospirillummagnetotacticum,* and so on can be used for bio-remediation of toxic metals like Fe (III) through reduction, where iron is actively taken by the cell, re-oxidized to hydrous oxide (low density) to Fe (III) oxide (ferrihydrite), which is of high density. The Fe(III) ions in the last step is reduced and magnetite is produced from dehydration within the magnetosome vesicles. An intracellular protein Ferritin accumulates the iron within the vesicles keeping it in non-toxic and soluble form. The nanoparticles produced have following characteristics like high purity, little crystalline defects, narrow size, mono-dispersive and so on *(*Aljarba *et al.,* 2022*).* The thermophilic bacteria can be an excellent tool for the extracellular synthesis of both gold and silver nanoparticles.

- **Synthesis of gold NPs by yeast strains**

The yeast *Hansenulaanomala* has the ability to donate electrons and can act as a catalyst in the case of bio-fuel. The reductants are extracted from the yeast and used as a reagent for the preparation of gold nanoparticles. However, very few yeast strains have been used in the investigation of gold nanoparticles *(*Zang *et al.,*2016)

- **Synthesis of gold NPs by fungi**

In this recent day, a wider variety of fungi have been exploited as they are found to be a potential player in the biogenesis of the nanoparticles like the gold. They are widely used as they secrete larger bulks of enzymes that have many advantageous applications and can be worked in the laboratory. The filamentous fungi have unique advantages over other microorganisms like bacteria and algae, as they have high metal tolerance and have the capability of *bio-accumulation*. Biological systems offer unique promising features to tailor nanomaterials with predefined properties. Fungi are the favorite choice of microorganisms due to the wide variety of advantages they offer over bacteria, yeast, actinomycetes1, plants, and other physio-chemical techniques. The use of fungi for the deliberate synthesis of gold nanoparticle is a fairly new and exciting area of research with considerable potential for further development.Fungi are attractive agents for biogenic synthesis of gold nanoparticles, because they offer high tolerance to

metals and are easy to handle. They also secrete large quantities of extracellular proteins that contribute to the stability of the nanoparticles. Advantages of fungal cultures over bacterial systems are that they provide good biomass production and do not require additional steps to extract the filtrate. Compared to synthesis using plants, the mycelial mass of fungi is more resistant to agitation and pressure, so it is more suitable for large-scale syntheses. Furthermore, by adjusting culture conditions such as time, temperature, pH, and quantity of biomass, among others, it is possible to manipulate the metabolism of fungi so as to obtain nanoparticles with the desired characteristics, such as specific size and morphology (*Zielonka* et al., *2017).*

- ***Parthenium hysterophorus* Linnaeus**

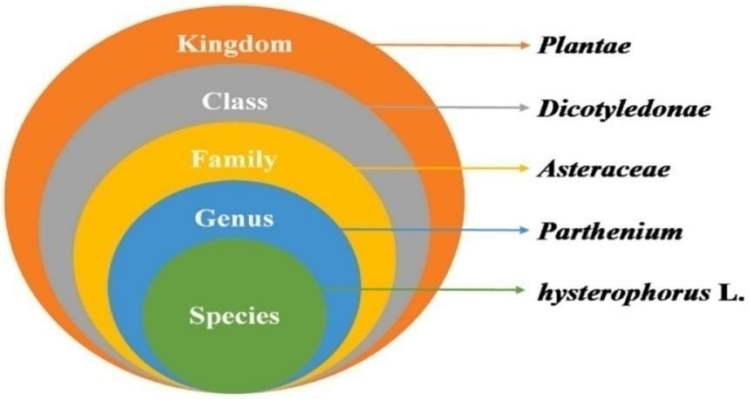


Fig. 3. Scientific classification of *P. hysterophorus*.

Figure 3: Scientific classification of Parthenium.hysterophorus

Parthenium hysterophorus L. (Asteraceae), commonly referred to as parthenium weed, is a fast growing, multi-branched herbaceous annual. The plant may flower as early as 35 days after germination *(*Navie *et al* 2012*)* and can grow to a height up to 2.5 m *(*Adkins *et al.,* 2014*).* It is also tolerant to drought and salt stress *(*Bajwa *et al.* 2017*).* Enduring low water content through a troubling drought avoidance strategy *(*Cowie *et al.,* 2020*).* The excessive reproductive ability of this weed facilitates its invasion within suitable habitats. The small white flowers typically produce five fruits, each containing a single achene and two attached sterile florets (hereafter referred to as a seed) *(*Adkins *et al,* 2014*)*. *Parthenium hysterophorus* is popularly known as “feverfew”, and is a member of the Composite family (M. A. Siddiqui *et al.,* 1978). All parts of the plant are reported to be used as bitter tonic, febrifuge, emmenagogue, antidyscentricetc *(*Pankaj *et al, .*2001*),* it is a herbaceous plant native to the tropical Americas (the Caribbean

islands and the lands bordering the Caribbean) and now grows in Mexico, Central America, South America, the West Indies, East Africa, most of the southern part of the United States, parts of Asia (especially India), and Australia. efforts have been made to manage the weed employing different methods such as mechanical, competitive replacement (allelopathy), chemical, and biological control methods *(*S. Kumar *et al.,* 2009).Approximately two million hectares of land in India have been infested with this herbaceous menace *(*Dwivedi *et al., 2009).*Since *P. hysterophorus* grows luxuriantly in many parts of the world, it is important to explore its beneficial uses if any. It is commonly known as 'altamisa', carrot grass, bitter weed, star weed, white top, wild feverfew, the "Scourge of India" and congress grass .It is a prolific weed belonging to Asteraceae family. Since *Parthenium hysterophorus* grows luxuriantly in many parts of the world, it is important to explore its beneficial uses if any. Chemical analysis of *Parthenium hysterophorus* has indicated that all its parts including trichomes and pollen contain toxins called sesquiterpene lactones (SQL*). (*Maishi *et al.,* 1998*)* reported that *P. hysterophorus* contains a bitter glycoside parthenin, a major sesquiterpene lactone. Other phytotoxic compounds or allelochemicals are hysterin, ambrosin, flavonoids such as quercelagetin 3,7-dimethylether, 6-hydroxyl kaempferol 3-0 arabinoglucoside, fumaric acid. P-hydroxy benzoin and vanillic acid, caffeic acid, p courmaric, anisic acid, p-anisic acid, chlorogenic acid, ferulic acid, sitosterol and some unidentified alcohols. The decoction of *Parthenium hysterophorus* has been used in traditional medicine to treat fever, diarrhoea, neurologic disorders, urinary tract infections, dysentery, malaria. Ethnobotanically, it is used by some tribes as remedy for inflammation, eczema, skin rashes, herpes, rheumatic pain, cold, heart trouble and gynecological ailments. *Parthenium hysterophorus* has been found to be pharmacologically active as analgesic in muscular rheumatism, therapeutic for neuralgia and as vermifuge *(*Maishi *et al., 1998)* Parthenin, the major constituent of the plant, exhibits significant medicinal attributes including anticancer property *(*Venkataiah *et al., 2003*). Methanol extract of the flowers showed significant antitumour activity and parthenin exhibited cytotoxic properties against T cell leukaemia, HL-60 and Hela cancer cell lines *(*Das *et al.,* 2007).Allelopathy can be used to increase crop production at minimal expenses and to diminish the current reliance on synthetic agrochemicals that degrade the environmental quality. The allelochemicals can be exploited as herbicides, insecticides, nematicides, fungicides and growth regulator. The

allelochemicals also provide defence against herbivorous predators. *(*Kisho*r et a.,.* 2010*)* prepared compost of *Parthenium hysterophorus* in 14 week Compost from this weed on application in soil enhanced its moisture level more than nitrogen, phosphorus and potassium (NPK) alone*. (*Javaid *et al.,* 2008) used *Parthenium hysterophorus* weed as green manure for maize and mung bean production. *Parthenium hysterophorus* being rich in N, P, K, Ca, Mg and chlorophyll content is ideally suited for composting. Addition of parthenium green manure to the soil enhanced organic matter and nutrient availability consequently root and shoot growth of wheat was increased. The parthenium plant residue showed inhibitory effects on an aquatic weed, water hyacinth, at a much lower level. It either inhibited growth of water hyacinth at lower doses or killed the plants at higher doses, resulting in clearing the water surface *(*pandey *et al.,* 1993*)* However, growth of wheat seedlings continued even at much higher levels of parthenium residue than the lethal dose for water hyacinth. The potential of *Parthenium hysterophorus* as low-cost raw material for xylanase production was studied by *(*Dwived *et al.,* 2009*)* hey investigated xylanase production from a mutant of *Penicillium oxalicum* in submerged fermentation. Considerably higher level of the enzyme production in medium containing *Parthenium hysterophorus* confirms the feasibility of using this cheap resource as an alternative carbon source to save costs of the enzyme production process.

Ancient, Indian medicinal practices include, Ayurveda, Siddha, Unani, Amchi and local health traditions that employ plants, provides a strong base for the safe treatment and effective management of wide range of diseases. A good example is the stem of the *Parthenium hysterophorus* is soaked in water used to relieve toothache and strengthen the gums. Boils and pimples may be removed by soaking ground roots in water. Juices obtained from its leaves offer relief from constipation and used on the eyes to treat insomnia *(*Marwat *et al.,* 2015*)*

Due to their influence on signal transduction in cell proliferation as well as on angiogenesis, various studies have demonstrated the chemo-preventive ability of P*arthenium hysterophorus (*Kumar *et al.,* 2014*).*In a country like India, uprooting and burning of *Parthenium hysterophorus* infested crop is a practice used to eradicate the weed. Thus, there is a need to develop green (eco-friendly) methods to manage and lessen the spread of the weed. *Parthenium hysterophorus* can be used for the green synthesis of nanoparticles that found applications in the medical and electronic field for

the synthesis of inorganic nonmaterial's *(*Parashar *et al., 2009).*The presence of poly-phenolics (act as a reducing agent) in *Parthenium hysterophorus* is a way to synthesize nanoparticles that are sustainable and free from chemical contaminants *(*Ahsan *et al.,* 2020*). Parthenium hysterophorus* should be seriously considered as a substrate for the production of biogas in India via anaerobic digestion, considering the abundance of this weed and large quantity of livestock. It can also be used as a flea-repellent for dogs and is a valuable source of potash, oxalic acids and High-quality protein (HQP) which can be used in animal feed *(*Mane *et al.,* 1986*).*

Characterization of gold nanoparticles

UV-Vis spectroscopy measurements

UV-vis spectroscopy uses the phenomenon of SPR, which enables the AuNPs to absorb light at a specific wavelength. Also, the absorption peak changes according to the size of nanoparticles*. (*Lou *et al.,* 2012*)* have used UV-vis spectroscopy for the characterization of AuNPs synthesized using the citrate reduction method. UV-vis spectroscopy is a conventional technique used for the determination of bioconjugation.

Scanning electron microscopy (SEM)

Scanning electron microscopy is a high-resolution method for estimating size, size distribution, shape, aggregation, dispersion (cryo-SEM), and crystallinity (electron backscattering detection). It may be used to analyze inorganic, organic, carbon-based, biological, and complex materials. Scanning electron microscopy is limited to the analysis of conductive or coated materials under non-physiological conditions. *(*Breaker *et al.,* 2014*)*. SEM has also been used for the surface studies of AuNPs.

Transmission electron microscopy (TEM)

Transmission electron microscopy measures size and size distribution and confirms the nonmaterial shapes with higher resolution compared to SEM. Aggregation, dispersion (environmental TEM), and crystal structure can also be determined by TEM. It can be used to visualize inorganic, organic, carbon-based, biological, and complex materials as spherical and equiaxial particles, tubes, flakes, rods, or fibers. TEM along with DLS to characterize AuNPs and their conjugates *(*Tara nova *et al.,* 2017).

Fourier transform infrared (FTIR)

Fourier transform infrared spectroscopy (FTIR) can provide fundamental information on the molecular structure of organic and inorganic components, and is one of the most versatile analytical techniques for the non-destructive, chemical characterization of geological samples, such as coal, shale, fluid and melt inclusions, silicate glass, minerals, and microfossils *(*D'Mello *et al.,*2017*).*

Dynamic light scattering (DLS)

DLS also called photon-correlation spectroscopy, which is a widely popular and highly adaptable analytical method applied in different fields of life and material sciences, as well as in industrial quality control processes. Dynamic light scattering (DLS) has become an indispensable technique of size determination for diverse materials: from sub-micron particles to protein formulations. DLS is non-invasive, requires little sample preparation as well as small sample amounts *(*Stapleton *et al.,* 2014*).* The quality of a measurement depends on instrument hardware, as well on the properties of the sample, such as size, contrast, concentration, and the presence of impurities.

Application of gold nanoparticles:

- As an anti-biotic, anti-fungal, and anti-microbial agent when added in plastics, coatings, nanofibers and textiles
- In nanowires and catalyst applications
- In therapeutic agent delivery
- To connect resistors, conductors, and other elements of an electronic chip
- In photodynamic therapy - When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells
- In various sensors, e.g., colorimetric sensor with gold nanoparticles can identify if foods are suitable for consumption
- As substrates to enable the measurement of vibrational energies of chemical bonds in surface enhanced Raman spectroscopy
- The scattered colors of gold nanoparticles are currently used for biological imaging applications
- Gold nanoparticles are quite dense, thus allowing them to be used as probes for transmission electron microscopy

- To detect biomarkers in the diagnosis of cancers, heart diseases, and infectious agents
- As catalysts in a number of chemical reactions
- For fuel cell applications

Anticancer activity of gold nanoparticles

A variety of biogenic AuNPs, with anticancer therapeutic property have been recently reported *(*Geetha *et al.,* 2013*).* Regarding to the poor bioavailability and selectivity and serious toxicity of gold-based anticancer complexes, the biogenic AuNPs without these deficiencies, would display a promising chemotherapeutic potential for cancer therapy *(*Zou *et al.,* 2015*).* High stability, low cytotoxicity, biocompatibility, and multi-functional potential offered by Au-NPs makes it potential candidate for drug delivery system *(*Eleraky *et al.,* 2020*).* It is well established that the Au-NPs with phytochemicals have been extensively used for their antiviral, anti-allergic, anti-inflammatory, antioxidant and antitumor properties. Nevertheless, Au-NPs have shown potential applications for the delivery of antitumor agent's, such as cisplatin, oxaliplatin and paclitaxel through the detection of DNA *(*Aljarba *et al.,* 2022).The Au-NPs synthesize from a variety of plant sources, have been applied in the treatment of different kinds of cancers such as breast cancer cells MCF-7, Hep2 and A549 cells. As such, several researchers aimed to develop low-cost, effective, eco-friendly Au-NPs to cure cancer and microbial infections *(* Rajeshkumar*. et al.,* 2021*).*

OBJECTIVE

Objectives

- Green synthesis of gold nanoparticles by using *Parthenium hysterophorus* leaf extract.
- Characterization of biogenically synthesized gold nanoparticles by UV-Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential.
- Investigation of the efficacy of synthesized gold nanoparticles against cervical cancer HeLa cells.

MATERIAL AND METHOD

Material and Methods

Chemicals

HiMedia, India; Merck and Sigma-Aldrich Co. (St. Louis, MO, USA) provided all the chemicals required for the study.

Plant Collection and extract preparation

Parthenium hysterophous plant was a kind gift from Dr Maqbool Ahmad Khan, Deputy Director of CCRMU, Kursi Road, Basha, Lucknow 226026. Healthy green leaves were collected rinsed properly with the still water to remove all the dust and unwanted visible impurities. The leaves were crushed with the help of pestle mortar and tris buffer was added in it. Take some ice cubes in the polypropylene molded tray and place pestle & mortar in it with plant extract and leave it for some time. Then again crush the extract and filter it with the help of Whatman filter paper in the centrifuge tube and then the tubes were placed in centrifuge at 6000 rpm at 4°C for 10 min. Then remove the pellet from the extract and take the supernatant in another centrifuge tube. Extract is stored in refrigerator for future purposes.

Parthenium hysterophous -mediated synthesis of gold nanoparticles

In vitro synthesis of AuNPs was performed by 3 ml of the prepared plant extract was taken in 20 ml of centrifuge tube and 1mM of gold chloride salt and PBS was added to the plant extract. Keep the reaction tube in incubator at about 37°C. After 48 h the different period the sample was ejected and analyzed on a bio spectrum-Kinetic spectrophotometer using a quartz cuvette having the path length of 1 cm to a affirm the synthesis of cannabis sativa encapsulated gold nanoparticle subsequently, the solution was filtered using a syringe with a filter having the pore size of 2 micrometer, the unbound proteins and phytochemicals where expelled using ethanol treatment for 30 minutes and utilized further characterization.

Characterization of gold nanoparticles

The transformation of gold salt into gold nanoparticles was investigated by using the Shimadzu UV-1601 dual beam spectrometer. This measurement has a special

resolution of one nanometer (200 nm to 800 nm). The technique is done on the basis of reducing metal salts to synthesize gold nanoparticles result in color change from light green to ruby red. Particle size analyser (Zetasizer Nano-ZS, Model ZEN3600, Malvern Instrument Ltd., Malvern, UK) was used to analyze the mean particle size of AuNPs. The diluted sample (0.5% w/v) was sonicated for 1 min. and taken in a low volume disposable sizing cuvette of 1.5 mL. The mean particle size was the average of triplicate measurement for a single sample. The zeta potential measures the colloidal stability of nanoparticles in a solution as previously described, that metal nanoparticles carry charge for capping agents, Zeta potential may also be used to assess the shielding or exposure of charged groups, as well as the concentration distribution of nanoparticles .

Cell Culture

The human cervical cancer cell line (HeLa) was purchased from National Centre for Cell Science (NCCS), Pune, India. The aforementioned *in-vitro* cytotoxic potential analysis of Prth-extract Prth-AuNPs was performed on HeLa cells using MTT assay. The cells were cultured in DMEM medium, supplemented with 10% FBS and 1% antibiotics containing 10,000 units/ml of penicillin, 10 mg/ml of streptomycin, and 25 µg/ml of amphotericin B in a humidified atmosphere containing 5% CO_2 at temperature 37°C. All the cell stocks were maintained in 25 cm^2 tissue culture flasks.

Assessment of cytotoxicity

To assess the cytotoxic effect of Prth-extract and Prth-AuNPs, HeLa cells were placed in 96-well plate with density of 1×10^4 cells per well and incubated in a humidified incubator with 5% CO_2 at 37°C for 24 h. Further the cells were treated with Prth-extract and Prth-AuNPs different concentrations in triplicates, and incubated for 24 h. After incubation, the media was discarded and 10µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (5 mg/mL in PBS) was added to each well. The plates were further incubated for 2 h in a CO_2 incubator. The resulting formazan crystals were solubilized in 100µL of DMSO. The extent of MTT reduction was measured spectrophotometrically at 595 nm using a Bio-Rad Elisa reader, and the cell survival was expressed as percentage over the vehicle. Experiments were conducted in triplicate. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50% (IC50). The IC50 values were determined with GraphPad Prism5 computer program.

Percentage cell viability was calculated as follows:

$$\% \text{ Cell viability} = \frac{¼ \text{ Absorbance of treated cells} - \text{Absorbance of blank}}{\text{Absorbance of untreated cells} - \text{Absorbance of blank}} \times 100$$

Measurement of morphological Alteration in HeLa cells

HeLa cells were pre-treated with different concentrations of each, Prth extract and Prth-AuNPs incubated for 24 h at 37°C in an atmosphere 5% CO_2. Post-incubation, the morphological changes in HeLa cells occurred in the all the treated groups were examined using an inverted phase contrast microscope (FLoid Imaging station, Thermofisher, USA).

RESULT AND DISSCUSSION

Result and Discussion

Parthenium hysterophorus extract mediated synthesis of AuNPs (Prth-AuNPs)

This study used *Parthenium hysterophorus* leaf extract as a reducing and capping agent, whereas1Mm gold chloride ($HAuCl_4$) served as the gold precursor. The synthesis of Prth-AuNPs is considered to be induced by the aqueous extracts reducing enzymes and capping agents, such as secondary metabolites. The creation of Prth-AuNPs was confirmed visually by a shift in the color of the extract from green to ruby red, indicating gold reduction.

Characterization of Prth-AuNPs

The phyto-constituents in *Parthenium hysterophorus* leaf extract reduced the gold salt ($AuCl_4$) into AuNPs and encapsulated the gold nanoparticle preventing the nanoparticles from the aggregating and providing stability to the Prth-AuNPs. The change in color from light green to ruby red indicated the successful synthesis of Prth-AuNPs and the result of SPR (surface plasma resonance) band confirm that at 530 and however there was no discernible peek for *Parthenium hysterophorus* leaf extract.

The technique of dynamic light extracting (DLS) was used to determine the average particle size and provide of the particle size distribution of Prth-AuNPs had an average particle size of 58.46 d nm as shown in figure. Furthermore, the Zeta potential of the prepared Prth-AuNPs was observed at the room temperature, to be a -22.2 mV, indicating the significantly high stability of the nanoparticles. When the aqueous dispersion of AuNPs was observed at room temperature no clumping or accumulation was observed. This was most likely due to the silver and a particle electrostatic repulsive effect. The nanoparticles are prevented from colliding because of this repulsion.

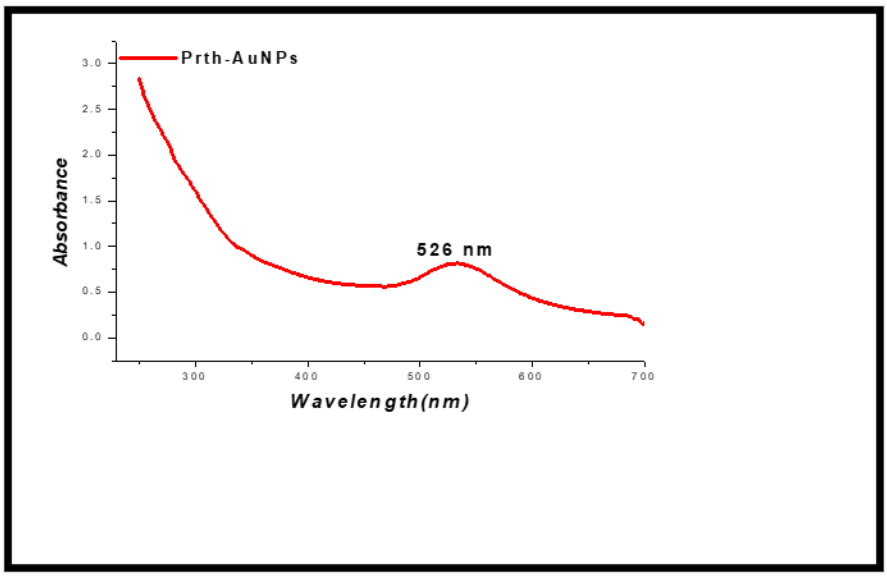


Figure-4: -. Characterisation of Prth-AuNPs under UV-Visible spectra (526 nm).

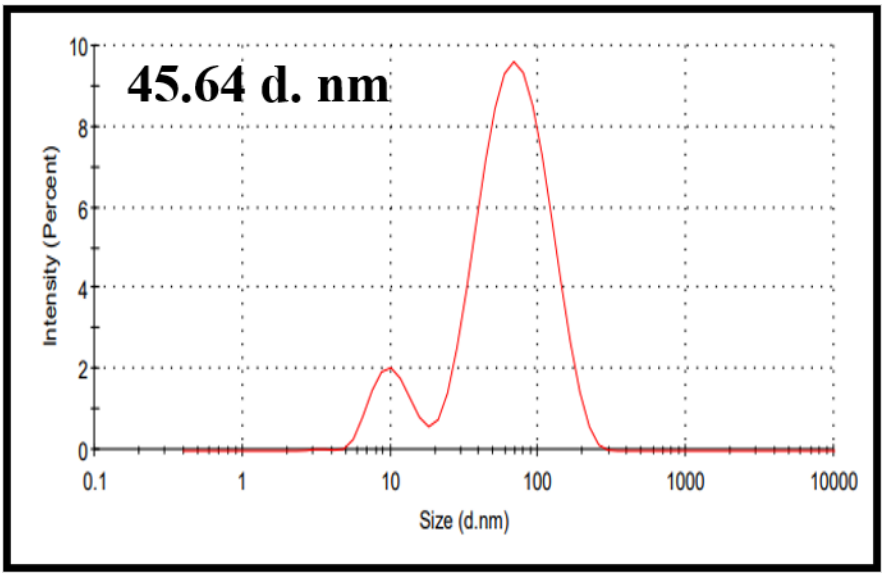


Figure- 5: DLS profile of Prth-AuNPs showing size of 45.64 d.nm.

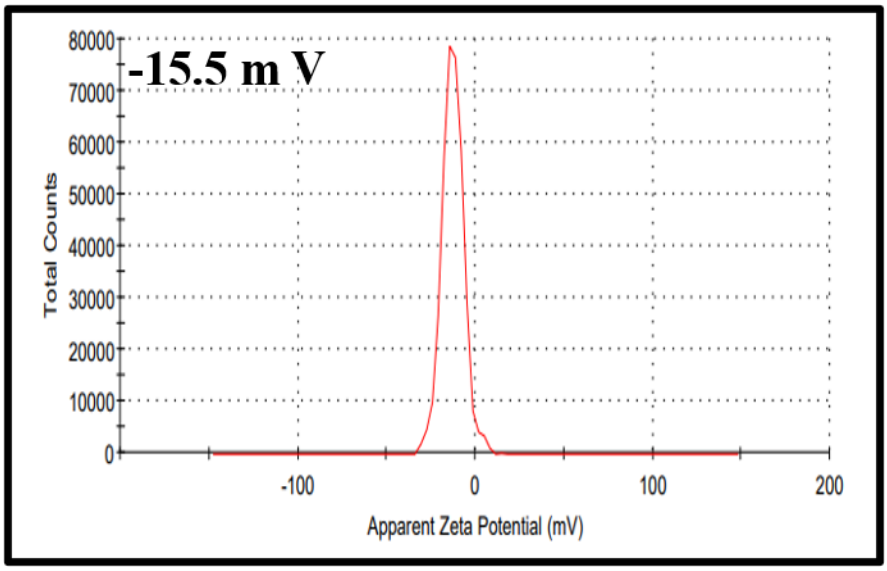


Figure-6: Zeta potential of Prth-AuNPs confirmed the stability at -15.5 mV

Determination of morphological alteration in the HeLa cells

Morphological analysis of the Prth-extract and Prth-Au NPs, treated HeLa cells was performed using a phase contrast microscope. A dose-dependent change in the cell morphology was observed in HeLa cells after treatment with Prth- extract (100µg/ml, 200µg/ml, 300µg/ml, 400 µg/ml) and Prth-AuNPs, (5µg/ml, 10µg/ml, 20µg/ml, 40 µg/ml) concentrations for 24 h. In the presence of different doses Prth-extract and Prth-Au NPs. HeLa cells showed round morphology with small shrinkage and nuclear condensation. A proportion of the cells revealed swelling, cell membrane lysis and disintegration of organelles, suggesting cytotoxicity in HeLa cell. These morphological changes in cervical cancer cells were more evident with the increase in the dose in AuNPs. In contrast, well spread flattened morphology was observed in untreated control cells.

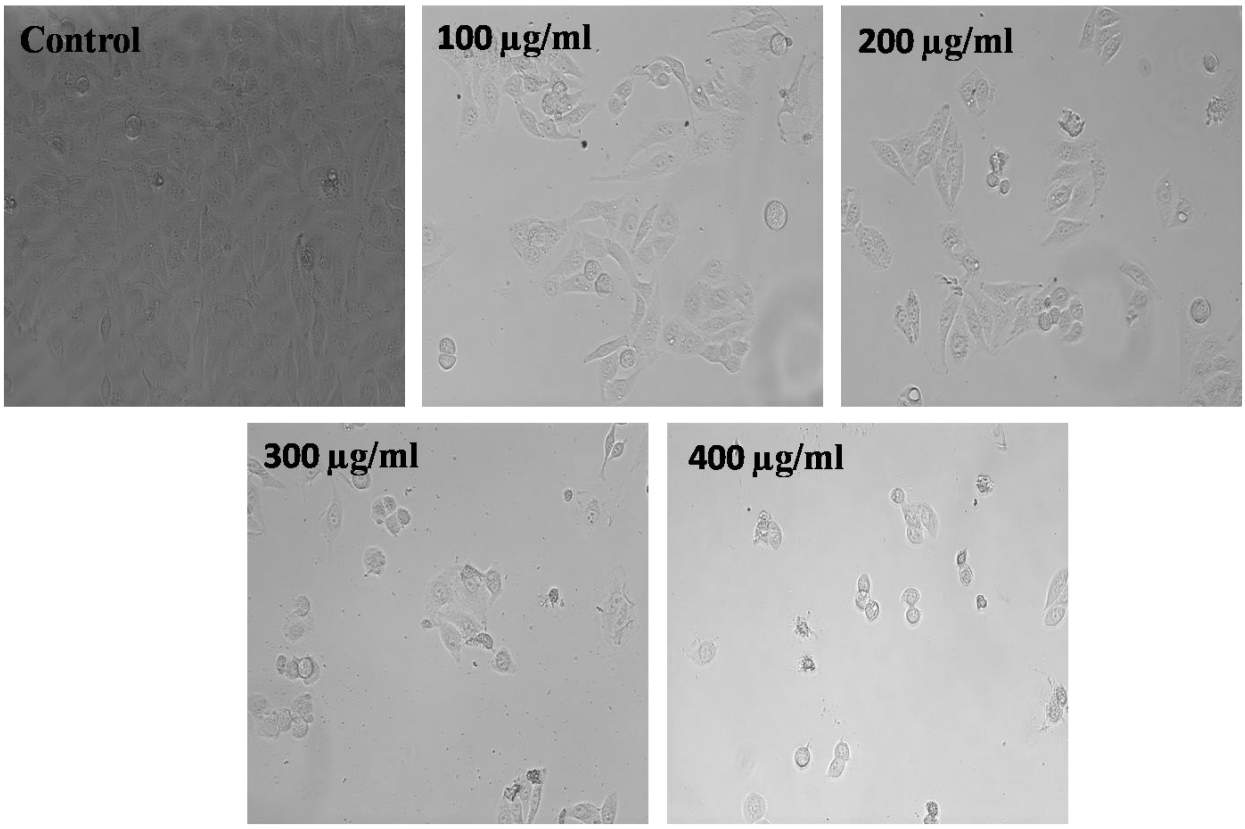


Figure:7- The phase contrast microscopy of HeLa cell treated with either vehicle control or different doses concentration of Prth-extract (100µg/ml, 200µg/ml, 300µg/ml 400µg/ml) for 24 h in a time- and dose- dependent manner. Image shown are representative of three independent experiments (Scale bar: 100µm; magnification: 20X)

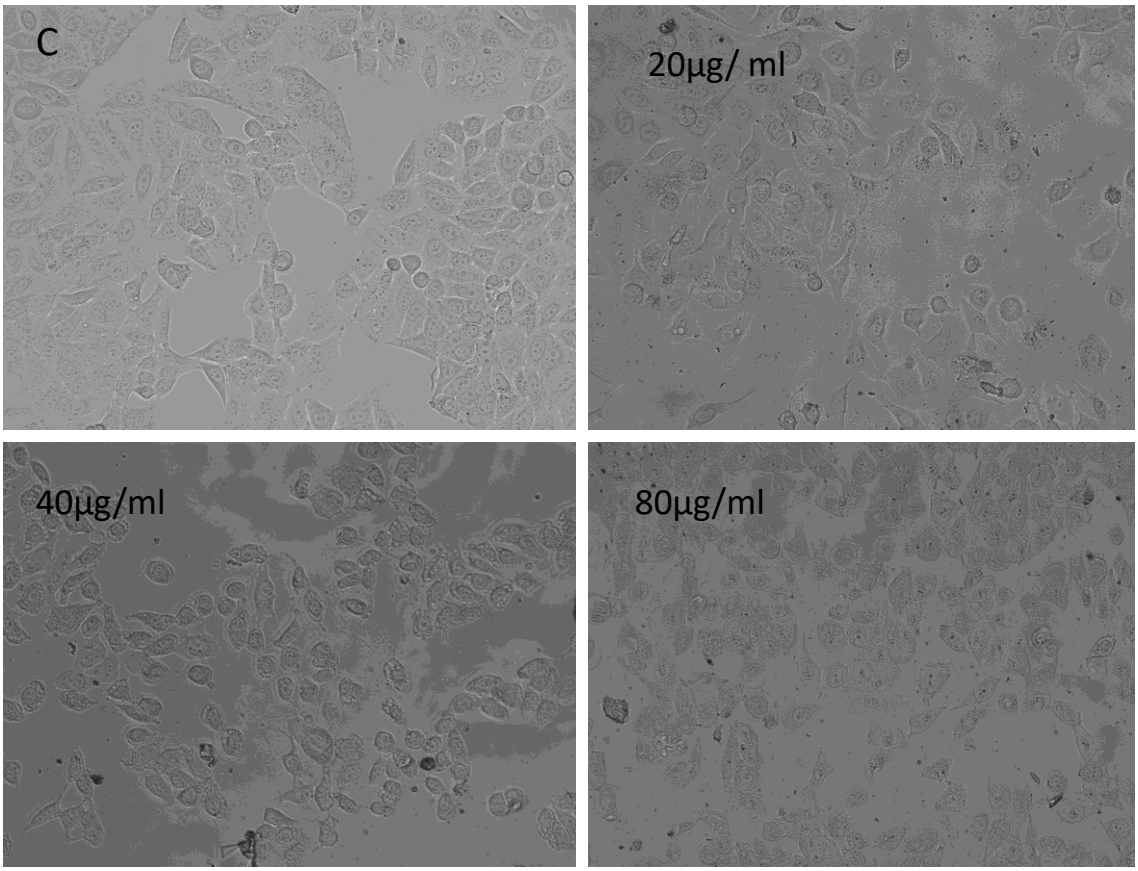


Figure- 8. Phase contrast micrographs of HeLa cells treated with either vehicle control or different doses of Prth-AuNPs (5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml) for 24 h in time- and dose -dependent manner. Image shown are representative of three independent experiments (Scale bar: 100µm; magnification: 20X)

In vitro cytotoxicity of Prth- extract and Prth-AuNPs

To evaluate the sensitivity of lung cancer cells to these drugs, HeLa cells were treated with different doses of Prth-extract and Prth-AuNPs, for 24 hours followed by MTT assay. Our results showed that, after 24 h of treatment, AuNPs at IC50 concentration of 203.00±1.13µg/ml reduced growth of HeLa cells by 50%, while inhibition of 50% viability of HeLa was observed at IC50 concentration of 25.43±1.09µg/ml Prth-AuNPs, respectively. AuNPs were found to be more cytotoxic for cervical cancer cells in comparison to pure extract and the effect was observed to be dose-and time-dependent.

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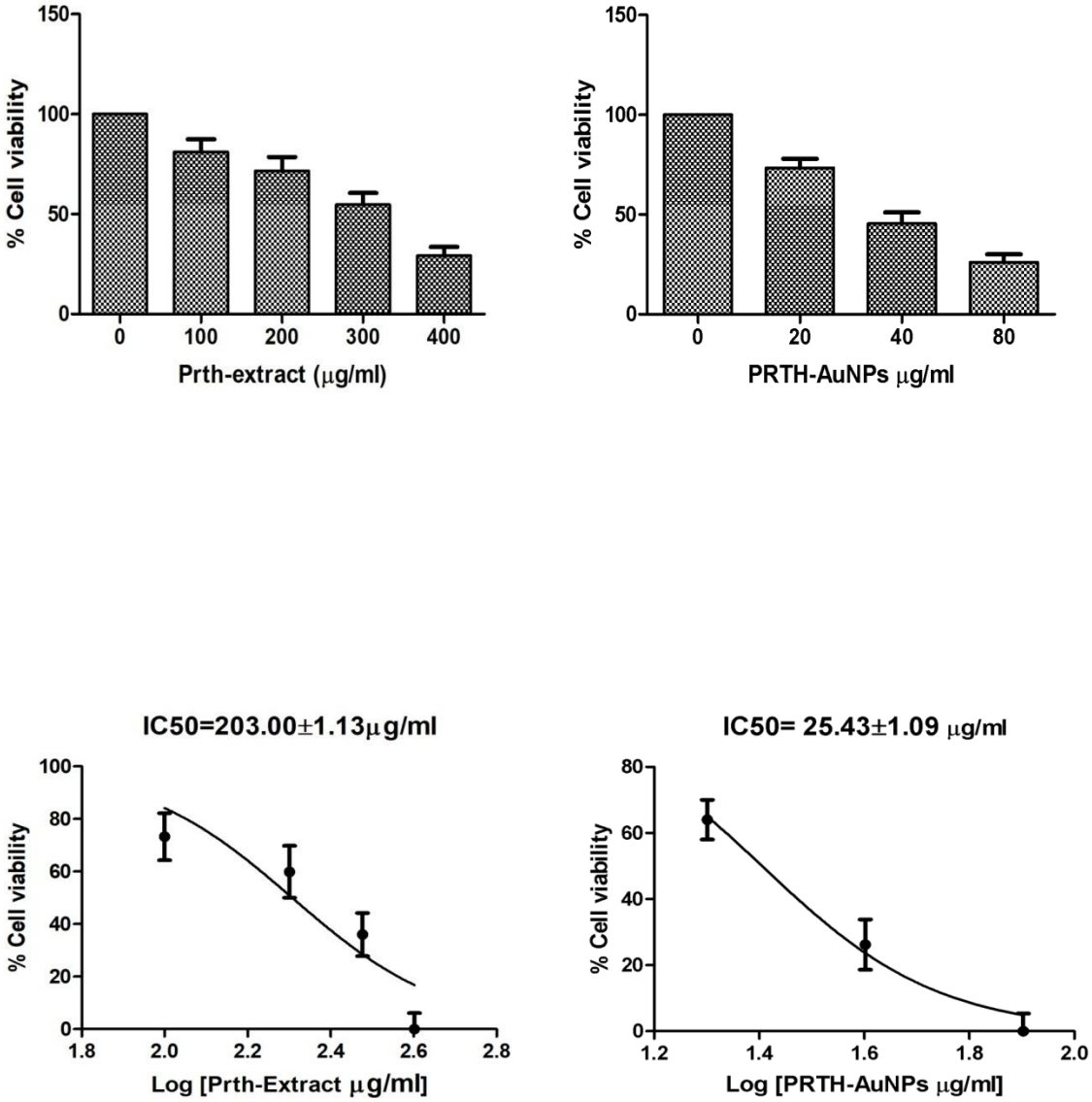

Figure-9: - Percent cell viability of HeLa cells treated with different doses of *Parthenium hysterophorus* (100-400µg/ml) as assessed by MTT Assay 24 h. Graph showed that *Parthenium extract* exhibited IC50 value 203.00±1.13µg/ml and 25.43±1.09µg/ml at 24 h, against HeLa cervical cancer cells. The result represented are the mean ±SEM of three independent experiment performed in triplicate.

CONCLUSION

Conclusion

In this study, we showed leaf extract mediated green synthesis of gold nanoparticles from plant *Parthenium hysterophorus* and their characterization, and anticancer analysis. This study investigates an efficient and sustainable route of AuNPs preparation from 1mM aqueous $HAuCl_4$ using leaf extracts of *Parthenium hysterophorus* plants. The AuNPs were characterized by UV-visible spectrophotometer, particle size analyzer (DLS) and Zeta potential.

This study comprehensively addressed synthesis, characterization, and bio-applications of gold nanoparticles, with special emphasis on therapeutic approaches for cancer using AuNPs. Recently, both academic and industrial research has explored the possibility of using AuNPs as a next-generation anticancer therapeutic agent, due to the conventional side effects of chemo- and radiation therapy. Although AuNPs play an important role in clinical research, several factors need to be considered, including the source of raw materials, the method of production, stability, bio-distribution, controlled release, accumulation, cell-specific targeting, and finally toxicological issues to human beings. The development of AuNPs as anti-angiogenic molecules is one of the most interesting approaches for cancer treatment and other angiogenesis-related diseases; it can overcome poor delivery and the problem of drug resistance.

Although AuNPs have been focused on therapeutic purposes, further research is inevitable in animal models to confirm the mechanisms and to gain a comprehensive picture of biocompatibility vs. toxicity of AuNPs. Finally, if we succeed in all these studies, it would help the researchers of the nanoscience and nanotechnology community to develop safer, biocompatible, efficient cancer or anti-angiogenic agents containing AuNPs. Eventually, to ensure the biosafety of the use of AuNPs in humans, studies dealing with biocompatibility of AuNPs and their interaction with cells and tissues are inevitable. Finally, the great concern is that the developing nanotechnology-based therapy should be better than available technologies, and it should overcome the limitations of existing treatment techniques. Finally, it has to provide a safe, reliable, and viable treatment of diseases with high accuracy in a patient-friendly manner.

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