A DISSERTATION ON

BIOLOGICAL SYNTHESIS OF GOLD NANOPARTICLES BY CANNABIS SATIVA AND ASSESSMENT OF THEIR ANTICANCER EFFICACY AGAINST CERVICAL CANCER HELA CELLS

SUBMITTED TO THE

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FOR THE
DEGREE OF MASTER OF SCIENCE
IN BIOTECHNOLOGY

BY

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. RUMANA SHABIH**, a student of M.Sc. Biotechnology (II Year, IV semester), Integral University has completed her four months dissertation work entitled *"**Biological synthesis of gold nanoparticles by Cannabis sativa and assessment of their anticancer efficacy against cervical cancer HeLa cells"*** successfully. She has completed this work from Department of Biosciences, Integral University, under the guidance of **Dr. Irfan Ahmad Ansari**

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

Dr. Snober S. Mir
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This is to certify that the study conducted by **Ms. RUMANA SHABIH** 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 script of the thesis has been written by the candidate herself. The thesis entitled is *"**Biological synthesis of gold nanoparticles by Cannabis sativa and assessment of their anticancer efficacy against cervical cancer HeLa cells"*** therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of Master of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow.

Date:

Place: Lucknow

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Rumana Shabih

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LIST OF ABBREVIATION

AuNPs	Gold nanoparticles
mM	milli Molar
CNTs	Carbon nano tubes
QDs	Quantum dots
DLS	Dynamic Light scattering
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
UV-Vis	ultraviolet visible spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
XPS	X-ray Photon electron spectroscopy
XRD	X-ray Diffraction
CNB	Cannabis sativa
µg	Micro gram

INTRODUCTION

Introduction

Nanotechnology is a multidisciplinary field originating from the interaction of several different disciplines, such as engineering, physics, biology and chemistry. New materials and devices effectively interact with the body at molecular level, yielding a brand-new range of highly selective and targeted applications designed to maximize the therapeutic efficiency while reducing the side effects. Liposomes, quantum dots, carbon nanotubes and super paramagnetic nanoparticles are among the most assessed nanotechnologies. The field of nanoparticles (NPs) is one of the avenues to nanotechnology that is associated with nanoscale materials with very small particles size ranging from 1 to 100 nm. NPs exhibit distinctive properties owing to their extremely small size and high surface area to volume ratio, which have attributed to the significant differences in the properties over their bulk Counterparts *(Singh et al., 2011).*Nanotechnology, in the simplest form of the word, typically encompasses components with at least one feature smaller than a few hundred nanometers*(Theis et al., 2006).*Bionanotechnology is the integration between biotechnology and nanotechnology for developing biosynthetic and environmental friendly technology for the synthesis of nanomaterials .Over the last decade, novel synthesis approaches/methods for nanomaterials (such as metal nanoparticles, quantum dots (QDs), carbon nanotubes (CNTs), graphene, and their composites) have been an interesting area in nanoscience and technology*(Su et al., 2014)* . Nanostructured metal oxides have already been extensively studied for their promising use in technology. This has resulted in development of numerous reproducible procedures for the synthesis of nanoparticles with desired characteristics like size, shape, morphology, defects in the crystal structure, monodispersity providing a rich background for research relevant to antibacterial applications. Characterization of these nanoparticles can be helpful in modifying and tuning their antibacterial and cytotoxic effects. For instance, it has been established that the antibacterial activity increases with decreasing the particles size *(Zhang et al., 2010)*. the synthesis technique employed is functional in determining the biological characteristics of a given nanoparticle.Due to the increased demand for various metallic and non-metallic nanoparticles over the past two decades, a wide range of physical and chemical techniques have been developed to produce nanoparticles of

different sizes, shapes, and compositions. Traditionally, nanoparticles have been synthesized and stabilized via physical and chemical techniques. The physical approach includes techniques such as laser ablation , lithography and high-energy irradiation *(Chen et al., 2001).* While the chemical approach uses techniques such as: chemical reduction, electrochemistry, and photochemical reduction *(Starowicz et al., 2006)*Conventional synthesis of nanoparticles can involve expensive chemical and physical processes that often use toxic materials with potential hazards such as environmental toxicity, cytotoxicity, and carcinogenicity *(Kulkarni & Muddapur, 2014)*. As a result, the presence of these toxic formation agents on the synthesized nanoparticles and potentially the nanoparticles themselves has prevented their clinical and biomedical application. Importantly, all these factors can be potentially controlled via biological mediated production. As a result, there is currently widespread interest in developing clean, reliable, biologically compatible, benign, and environment-friendly green processes to synthesize nanoparticles*(Ahmad & Senapati, 2003).* In recent years, biological synthesis has emerged as an attractive alternative to traditional synthesis methods for producing nanoparticles. Biosynthesis involves using an environment-friendly green chemistry based approach that employs unicellular and multicellular biological entities such as actinomycetes , bacteria *(Mukherjee et al., 2001)*, fungus *(Philip, 2010),* plants *(Knez et al., 2004)*, viruses , and yeast *(Smijs & Pavel, 2011).*Among nanosized metal NPs , Au NPs has gained much more attention,because of their unique distinct features: inert, biocompatible, and especially due to low toxicity.Gold nanoparticles (AuNPs) have been widely explored and are well-known for their medical applications. Chemical and physical synthesis methods are a way to make AuNPs. In any case, the hunt for other more ecologically friendly and cost-effective large-scale technologies, such as environmentally friendly biological processes known as green synthesis, has been gaining interest by worldwide researchers.The international focus on green nanotechnology research has resulted in various nanomaterials being used in environmentally and physiologically acceptable applications. Several advantages over conventional physical and chemical synthesis (simple, one-step approach to synthesize, cost-effectiveness, energy efficiency, and biocompatibility) have drawn scientists' attention to exploring the green synthesis of AuNPs by exploiting plants' secondary

metabolites.The use of green synthesized AuNPs in the treatment of cancer by utilizing phytochemicals found in plant extracts.Plant extracts contain various metabolites or organic compounds (alkaloids, flavonoids, proteins, polysaccharides, cellulose, and phenolic compounds) and secondary metabolites, which are utilized for nanoparticle synthesis*(Marslin et al., 2018).* Synthesized AuNPs were initially identified in the change in reaction color (formation of red color) through UV-vis spectrophotometer analysis.DLS and Zeta potential confirmed the crystalline structure of gold nanoparticles, and the size, shape and distribution of nanoparticles were visualized by TEM image. AuNPs synthesis through this green method can contribute to other fields such as green photocatalyst, drug delivery, anti-microorganism, adsorbent, detector, and green separation science and technology*(Kumar et al., 2014).* In recent years, it has been observed that nanomaterials, such as gold nanoparticles (AuNPs), are of great interest to humans due to their wide range of uses in agriculture, remediation, medicine, health aspects, industry, pharmaceuticals, etc. The use of plant-derived AuNPs has brought significant advances in cancer diagnosis and treatment, although some work in this area began mainly a few decades ago .Gold nanoparticles (AuNPs) have attracted a lot of interest in cancer detection and diagnostics because of their intrinsic properties .*(Gong et al., 2020)*

REVIEW OF LITERATURE

Review of literature

Nanotechnology

Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale. The National Nanotechnology Initiative (NNI) in the United States define Nanotechnology as “a science, engineering, and technology conducted at the nanoscale (1 to 100 nm), where unique phenomena enable novel applications in a wide range of fields, from chemistry, physics and biology, to medicine, engineering and electronics”*(Bayda et al., 2019)*. The prefix ‘nano’ is referred to a Greek prefix meaning ‘dwarf’ or something very small and depicts one thousand millionth of a meter (10−9 m). We should distinguish between nanoscience, and nanotechnology. 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 & Soelaiman, 2005) .* Nanotechnology provides a structural analysis tool on the most important scale of organizational structure, atomic and cellular levels, and designs and manufactures synthetic biomaterials in nanoscale with new treatments and other emerging materials.Today many novel materials with high strength, low weight, and high chemical resistance are now available and assembled under nanomaterials *(Abraham, 2012),* nanotubes (carbon nanotube (CNT)), nanowires (light emitting diode (LED), nanocrystals, and nanocatalysts*(Ezema et al., 2014)*. Dr. Butt also reported that the widespread use of nanotechnology in various fields includes but is not limited to the following:

Energy - as in solar panels, fuel cells, batteries

Self-defense - such as in the production of special items

Medicine / health - such as anti-cancer drugs, implants, dental implants, diagnostic nerves

Environment and agriculture - such as water purification, animal pesticides, plant quality, pesticide nanocapsules, pesticides, pesticides and insect repellents, anti-toxicants, and filter.

Also, nanotechnology is now being adopted in the production of aerospace components such as nanocomposites - to improve its light weight and high energy properties and its lighting systems - using LEDs, popularly known as low-energy saving bulbs.Nanotechnology has transformed our daily lives in many ways, including in matters related to energy, the environment, and medicine. In terms of medicines, nanomaterials provide new diagnostic tools using imaging and diagnostic applications, *(Giljohann & Mirkin, 2009)*and are very popular, acting as drug delivery vehicles or treatment agents to achieve better and safer treatment results.*(Papasani et al., 2012)*Nanotechnology has made a profound impact on medical imaging and diagnostics. In the illustration, advances in nanotechnology have led to the clinical interpretation of iron oxide NPs as distinct MRI agents.*(Saksena et al., 2006)*Nanotechnology has been identified as one of the key technology-enabled technologies affecting all industries, including the food industry .It currently plays an active role in the development of new and innovative programs in the field of agriculture, food and food (called agri / food / feed). The most common applications are nano-encapsulated agrochemicals (such as nano-pesticides, fertilizers) and food additives / nutrients (nano-nutraceuticals), antimicrobials / biocides and active / intelligent packaging. *(Aschberger et al., 2015)*Information from recent list EFSA reveals 276 applications for agri / food / feed nanotechnology applications in the market and many more that are still being developed. *(Peters et al., 2014)*

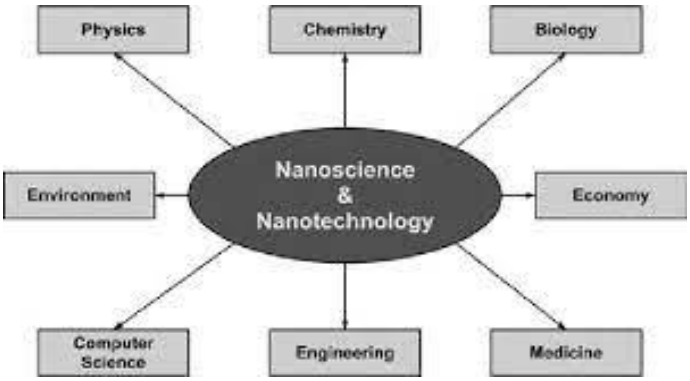

Figure.1-Multidisciplinary Areas of Nanotechnology

Nanoparticles

The rapid development of nanotechnology for biological purposes has had a profound effect on medicine. Nanotechnology enables the design and modification of materials at nanometer scale, thus allowing for the development of new tools for the treatment, diagnosis, monitoring, and control of biological systems. NPs have specialized physical and chemical properties that have been improved compared to a wide range of related materials. These properties include a high-to-volume ratio and a unique quantum size effect due to certain electronic properties. In addition to their structure, the properties of NPs depend on their size and shape *(Core, 2012)*.In general, in order to detect dispersed NPs and facilitate their incorporation into cells, it is necessary to control their size and shape and thus reduce fusion *(Chithrani & Chan, 2007).*

Types of nanoparticles

In terms of their chemical compounds, NPs can be divided into three main groups: organic nanoparticles (liposomes and polymers), inanimate nanoparticles (metals, metal oxide, ceramic, and quantum dots), and carbon-based nanoparticles *(Matteis & Rinaldi, 2018).*

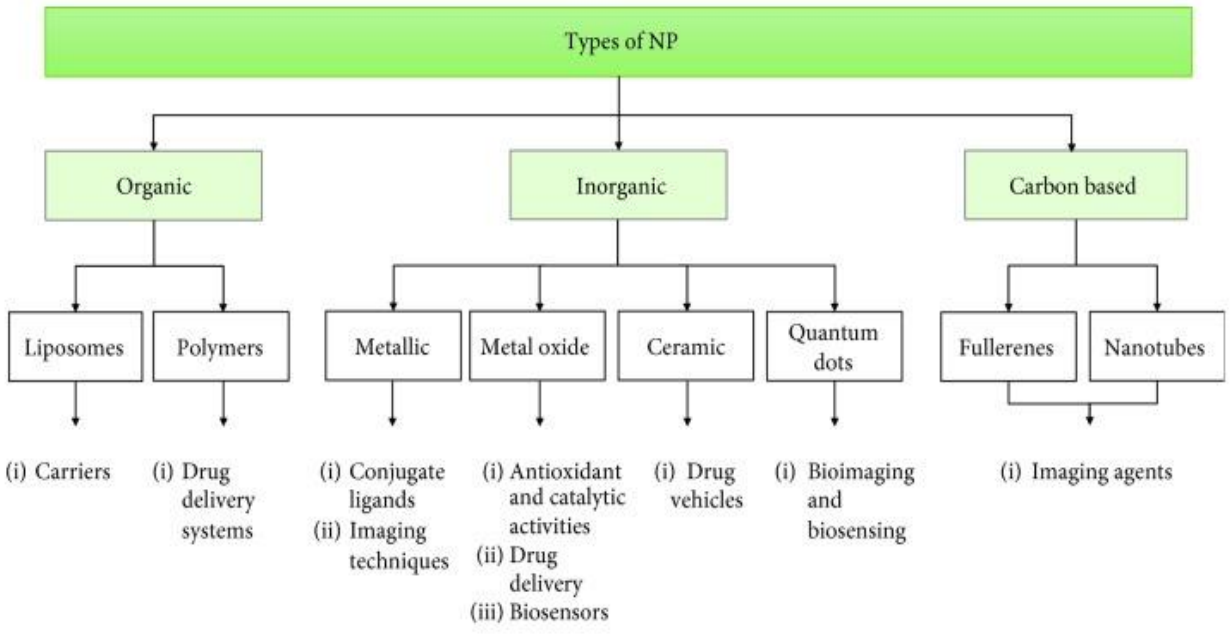

Figure.2. Types of Nanoparticles

Liposomes nanoparticles

These are circular vesicles with a membrane made of lipid bilayer that contains a liquid substance. Liposome synthesis is highly dependent on the following parameters: (a) the physicochemical properties of the substance to be absorbed and those of liposomal compounds; (b) the type of area in which the lipid vesicles dissolve, the binding of the substance, and the potential toxicity; (c) additional procedures involved during the manufacture, use, or delivery of vesicles; (d) dispersion, size, and shelf life of vesicles for intended use; and (e) duplicate-to-batch duplication and the potential for mass production of safe and effective liposomal products. Liposomes can be synthesized by incorporating sonicating amphipathic lipids, such as phospholipids, into water. Liposomes are widely used in chemotherapeutic drugs for the treatment of cancer. They can also include a high amount of active ingredients, including medications or dietary supplements. Liposomes are highly potent in nanomedicine, as well as in the food and cosmetics industries, due to their high compatibility with biodegradability.*(Panahi et al., 2017)*

Polymeric nanoparticle

Most polymeric nanoparticles are known for their decay and biocompatibility, making NPs widely used in drug delivery systems *(Patel et al., 2012)*. This type of nanoparticle can be synthesized from natural polymers, such as chitosan, or synthetic polymers, such as polylactides (PLA), poly (methyl methacrylate) (PMMA), or polyethylene glycol (PEG) . Polymeric nanoparticles can be prepared in a variety of ways, including two-step process based on emulsification, emulsification-solvent evaporation, emulsification-solvent diffusion, and emulsification-reverse salting-out. Additionally, there are similar steps to single-step procedures that include nanoprecipitation, dialysis and supercritical fluid technology. Among the techniques used to analyze further structures, we can find energy dispersive spectroscopy (EDS), zeta power (ζ-force), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), and Raman. These techniques reveal the chemical composition of the polymeric nanoparticle surface and its surface performance. However, using only a few techniques is possible to detect morphology and posture. Finally, it is important to consider that, in order to improve the effectiveness of

drug loading and increase drug release, the interaction of polymer compounds, as well as the type of polymer and its physicochemical properties, should be considered.

Metallic nanoparticles

These include precious metals (gold or silver) and magnetic metals (iron oxide or cobalt and ferrites containing manganese doped). Metal nanoparticles such as gold (Au) have distinct electronic and optical properties and are non-toxic and non-bio-compliant, and can be replaced by other biomolecules due to their poor charge. The gold surface provides an excellent opportunity to synthesize ligands such as proteins, oligonucleotides, and antibodies containing active groups such as phosphines, thiols, mercaptans, and amines, which have a high gold affinity. Gold nanoconjugates combined with highly developed gold nanoparticles of plasmon resonance are used in imaging techniques for various diseases *(El-Sayed et al., 2005).* In fact, El-Sayed et al. established the use of gold nanoparticles (AuNPs) for cancer imaging by choosing to transport AuNPs to the nucleus of the cancer cell, thus highlighting the importance of these nanoparticles in biomedicine.

Metal oxide nanoparticle

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 iron oxide (Fe3O4), titania (TiO2), zirconia (ZrO2), and more recently, ceria (CeO2) For example, titania nanoparticles have been implanted in medical applications because of the biocompatibility of its environment, as well as ceria nanoparticles is a matter of increasing attention due to their catalytic and antioxidant content, allowing them to act as antioxidants and anti-inflammatory agents *(Celardo et al., 2011)*. TiO2 is widely studied due to its biocompatibility, chemical stability, and physical properties, which provide important applications, for example, such as biosensor. Some metal oxide nanoparticles have a growing interest in their biomedical applications which may be cerium oxide (CeO2) nanoparticles or nanoceria. Nanoceria has a unique ability to switch between oxidation regions, thus enhancing its use in oxidative stress-related disorders. Studies have shown that effective use of other nanoparticles, such as

gold nanoparticles, in drug delivery and bioimaging. One important feature of gold nanoparticles is that their surface needs to be modified to protect them from biological systems, as they can easily dissolve in water and acidic solutions. In addition, in order for gold nanoparticles to be applied to fluorescence in the image, they first need to be removed, as the AuNPs bandwidth is in the UV environment and UV light cannot penetrate the tissues and can be harmful to cells and tissues.

Quantum dots

These are nanoparticles made of semiconductor materials with fluorescent properties. Typically, quantum dots (QDs) include semiconductor core (e.g., cadmium-selenium (CdSe), cadmium-tellurium (CdTe), indium-phosphate (InP), or indium- arsenate (InAs), covered with a shell. (e.g., zinc sulfide (ZnS)) to improve their visual and physical properties and prevent leakage of heavy toxins. These nanoparticles are widely used in bioimaging techniques and biosensing. However, these uses require that they be synthesized with biomolecules, such as proteins, peptides, or oligonucleotides, which enable them to bind to specific sites *(Xing et al., 2009).* The biocompatibility of QDs is critical to their biological and biomedical performance. Generally, biocompatible QDs can be obtained using three different mechanisms: (1) biomimetic synthesis, through or utilization of synthetic molecular structures or biomolecules (nucleic acids, peptides, proteins, and enzymes) as models; (2) biosynthesis, using organic matter in bioreactors; or (3) modify the QD surface taken from a chemical compound. The biosynthetic method provides a raw way to prepare compatible QDs without producing toxic products or aggressive reactions, while the modification method can create high QY on a large scale. One of the most important QDs is quantum gold dots (GQD), which have structures similar to those of gold nanoparticles; However, unlike other QDs, they do not show fluorescence. Instead, they have colorimetric properties caused by surface plasmon resonance (SPR) depending on solvency, composition, particle size and ligand, spatial function, dielectric properties, medium, and agglomeration, which make them very helpful in finding a biological application system, such as DNA. sequencing, hybridization testing, genetic disruption, flow cytometry, and immunoblotting.

Carbon based nanoparticles

This includes fullerenes and nanotubes. Fullernes novels carbon allotropes have a polygonal structure specially made up of 60 carbon atoms Carbon nanotubes are usually produced from the chemical vapor deposition of graphite. There are two categories of carbon nanotubes: single-walled (SWCNT) and multiwalled (MWCNT), the latter showing strong antimicrobial properties [53]. Carbon-based nanoparticles are considered to be of interest in biomedical applications due to their physical properties, which include better electrical conductivity and better mechanical strength, but they do not decompose and require environmental modification, as they have a strong tendency to form large aggregates *(Vardharajula et al., 2012).* Carbon nanotubes (CNTs) have outstanding properties, which is why they are used as labels and imaging agents. In fact, CNTs have a change in visual acuity near the infrared (NIR), making them useful for biological and cellular tissues, as the NIR has a lower concentration of excitement and greater penetration depth.

Ceramic nanoparticles

These are inorganic compounds with hollow features that have recently emerged as drug vehicles. They are able to transport molecules such as proteins, enzymes, or drugs without inflammation or jeopardize their porosity due to the external effects of pH or temperature. The most commonly used components in ceramic nanoparticles are silica and aluminum. However, the origin of these nanoparticles is not limited to these two; in fact, they can be made of a combination of metallic and nonmetallic materials. For example, CeO2-capped mesoporous silica nanoparticles (MSN) have been developed to act as transport vehicles by releasing β-cyclodextrin into lung cancer cells *(Xu et al., 2013)*. There are a variety of ceramic building materials with many applications, including minerals for clay, cement, and glass. Biocompatible Ceramics, also known as bioceramics, is mainly used for orthopedics, dentistry, and other medical applications. Bioceramics has a good balance of biocompatibility, hydrophilicity, osteoconductivity, biodegradability, and reabsorbability. 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 used in nanomedicine, orthopedics, bone regeneration, dentistry, and tissue development, in addition to other biomedical uses in the human body.

Gold nanoparticles

Gold nanoparticle chemistry and physics has emerged as a broad new subdiscipline in the domain of colloids and surfaces. The unusual optical properties of small gold particles, their sizedependent electrochemistry, and their high chemical stability have made them the model system of choice for exploring a wide range of phenomena including self-assembly, biolabeling, catalysis, electron-transfer theories, phase transfer, DNA melting and assays, and crystal growth. These nanoparticles (NPs) when stabilized or protected by a shell of thiolate ligands display good stability toward aggregation and other modes of decay, which enables attempts at isolating different NP sizes and the exploration of how NP properties depend on size (including quantization effects). NPs with fewer than 300 Au atoms can display distinct optical and electronic properties compared to the bulk metal. The thiolated NP stability further enables treating the ligand shell as a chemical platform that can be manipulated to exhibit desired reactivities, polyfunctionalization, and optical properties. The consequence for the past couple of decades has been a very active field of basic nanoscience research and applications of these NPs. An important aspect of Au NPs has been the breadth of their impact; applications range from photonic device fabrications, to sensing of organic and biomolecules, to charge storage systems.Spherical AuNPs possess useful attributes such as size- and shape-related optoelectronic properties,*(Limosani et al., 2022)* large surface-to-volume ratio, excellent biocompatibility, and low toxicity. These properties make AuNPs an important tool in bionanotechnology*(Zeng et al., 2014).*The ease of AuNP functionalization provides a versatile platform for nano biological assemblies with oligonucleotides, antibodies, and proteins*.(Calzolai et al., 2010)* Bioconjugates of AuNPs have also become promising candidates in the design of novel biomaterials for the investigation of biological systems*.(Jamison et al., 2011)*The versatility of AuNPs has provided useful materials for a range of biomedical applications . 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., drugs and targeting agents)*(Khan et al., 2011).*

Important physical properties of AuNPs include surface plasmon resonance (SPR) and the ability to quench fluorescence. Spherical AuNPs exhibit a range of colors (e.g., brown, orange, red and purple) in aqueous solution as the core size increases from 1 to 100 nm, and generally show a size-relative absorption peak from 500 to 550 nm.*(Jain et al., 2006)*

Methods for synthesis of AuNPs

- **Chemical methods**

Generally, the preparation of AuNPs by the chemical reduction method includes two main parts: (1) reduction by agents, for instance borohydrides, amino boranes, formaldehyde, hydrazine, hydroxylamine, polyols, citric and oxalic acids, sugars, hydrogen peroxide, carbon monoxide, sulfites, hydrogen, acetylene, and ono electronic reducing agents including electron-rich transition-metal sandwich complexes; (2) stabilization using agents, for instance trisodium citrate dihydrate, sulfur ligands (in particular thiolates), phosphorus ligands, oxygen-based ligands, nitrogen-based ligands (including heterocyclic compounds), dendrimers, polymers and surfactants (in particular, cetyltrimethylammonium bromide (CTAB)). To avoid the aggregation of the particles , some kind of stabilizing agent is usually added .*(Zhao et al., 2013)*

i. Turkevich method

Turkevich *et al*. 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 .*(Turkevich et al., 1951*)Frens further refined this method by changing the gold-to-citrate ratio to control particle size.*(Frens, 1973)*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. These citrate-stabilized AuNPs can undergo irreversible aggregation during functionalization process with thiolate ligands. Several strategies have been

developed to conquer this problem including using a surfactant, Tween 20, prior to the modification to prevent aggregation , or using thioctic acid as an intermediate *via* a two-step functionalization. *(Lin et al., 2004)*However, the requirement for high dilution makes large scale production challenging.

ii. The brust-schriffin method

Brust and Schriffin achieved a breakthrough in AuNP synthesis in 1994 by creating organic soluble alkanethiol-stabilized AuNPs through a biphasic reduction protocol using tetraoctylammonium bromide (TOAB) as the phase transfer reagent and sodium borohydride ($NaBH_4$) as the reducing agent .*(Brust et al., 1994)* This methodology produces low dispersity AuNPs from 1.5 to 5 nm by varying the reaction conditions such as gold-to-thiol ratio, reduction rate, and reaction temperature. These alkanethiol-protected AuNPs possess higher stability when compared to most other AuNPs due to the synergic effect of the strong thiol-gold interactions and van der Waals attractions between the neighboring ligands.*(Love et al., 2005)*These nanoparticles can be thoroughly dried and redispersed in solution without any aggregation making them excellent precursors for further functionalization .

- **Physical methods**

A number of advantageous characteristics of spherical AuNPs have been identified, including size- and shape-related optoelectronic capabilities, a high surface-to-volume ratio, great biocompatibility, and minimal toxicity. It was found that contact angle heavily relies on the nanoparticle size. According to the results, the contact angle for de-ionized water droplets ranged from 24° to 67° and for DEG (droplet-based electricity generator droplets), it ranged from 15° to 60°, for nanoparticle sizes that ranged from 14 to 620 nm. AuNPs exhibit several significant physical features, including surface plasmon resonance (SPR) and the ability to quench fluorescence. In aqueous solution, spherical AuNPs exhibit a spectrum of colors (e.g., brown, orange, red, and purple) as the core size grows from 1 to 100 nm, and often exhibit a size-relative maximum absorption between 500 and 550 nm. Furthermore, particles with high charges can cause double layers to

form in aqueous environments, and they can be discrete, dispersed, or suspended in the solution.

As opposed to the bulk shape, the energy levels of electrons in a substance in nano-form are not as continuous. The containment of the electronic wave function in up to three physical dimensions separates them. This causes a change in surface area and electron containment; the change in material properties is controlled in the same way that melting point, fluorescence, electrical conductivity, and magnetic permeability are *(Teimouri et al., 2018*). Ion coaters are an easy and direct method for generating uniform gold nanoparticles with a narrow size distribution by combining an ion coater on glycerin with a viscous liquid capture medium. A low-cost, low-energy synthesis technique that does not require additives or reducing/stabilizing agents. It is based on a physical low vapor deposition method rather than the conventional hydration process of chemical reactions in liquids. The surface plasmon resonance peak appeared at 530 nm in the absorption spectrum during the formation of gold nanoparticles; the red-shift with increasing particle size indicated that gold nanoparticles were successfully developed using the ion coater *(Lee et al., 2018*). Recently, researchers have concentrated on novel methods for synthesizing various shapes and sizes of controllable particles. There are optical physical characteristics associated with AuNP anatomy and physiology *(Elahi et al., 2018).*

- **Biological methods**

The biological synthesis of nanoparticles is a safe, dynamic, and energy efficient method of producing nanoparticles. This approach comprises a range of biological resources ranging from prokaryotes to eukaryotes to synthesize NPs in vivo. Metabolites (proteins, fatty acids, sugars, enzymes, and phenolic compounds) found in these sources play a significant role in both the bioreduction of metallic ions to NPs and their stability. AuNPs generated biologically are more stable than those generated using other methods. AuNPs can efficiently manufacture from chemical routes, but the main risk is the generation of by-products (secondary product) that are hazardous to human health and the environment. New routes for the production of safe nanoproducts are therefore being intensively explored by many biological systems, such as plants, bacteria, yeasts, and fungi, for the manufacture of AuNPs (*Teimouri et al., 2018)*.

- **Synthesis by fungus**

Green synthetic approaches for the preparation of various types of nanoparticles are critical for the preservation of long-term growth. Because of the scalability and cost-effectiveness of fungal growth on an industrial scale, extracellular or intracellular extracts of fungi are suitable materials for the synthesis of metal nanoparticles. Fungi can produce gold nanoparticles in one of three ways: extracellular, fungal autolysate, or intracellular. The size and distribution of the fungi differ depending on the strain and the experimental conditions (*Molnár et al., 2018)*. Fungi have an advantage over other microorganisms in that they can produce a large number of extracellular enzymes capable of reducing metal salts to nanoparticles. Fungi can also be easily prepared in the laboratory as well as on a large scale, as mycelia can withstand harsh conditions in bioreactors. Marine endophytic fungi have been found coexisting with marine algae. Several Scholars have been able to synthesize antioxidant gold nanoparticles from *Penicilliumcitrinum*, an endophytic fungus isolated from the seaweed *Sargassumwightii*, in recent years . The advantage of using *M. phaseolina* to create gold nanoparticles is that its oxidoreductase activity is higher than that of other fungal species, which is economically useful since less enzyme is needed for the generation of gold nanoparticles. Endophytic fungal isolates are grown for 21 days at 25 °C-28 °C in potato dextrose broth (PDB). In PDB, mycelial biomass is created, then extracted by filtration, and the traces of the media components are removed by washing with distilled water. Incubate the biomass in 100 ml of distilled water for 48 h at room temperature. Gold nanoparticles are generated by combining a 1 mM $HAuCl_4$ aqueous solution with a fungal suspension filter *(Osonga et al., 2020).* The solution was then recovered using centrifugation (10,000 rpm for 10 min). Finally, the filtered gold nanoparticles were washed with distilled water. The initial stage of myco-synthesis of gold nanoparticles is detected by a visual color change in the reaction flasks and verified by UV–Vis spectroscopy *(Sreedharan et al., 2019).* pH, cell growth rate, and temperature all had an effect on the morphology and size of gold nanoparticles during development. The optimal temperature for the production of gold nanoparticles was identified by adjusting the incubation temperature of the cell-free filtrate from 28 to 55 °C. The ideal pH for gold

nanoparticle formation was identified by changing the pH of the cell-free filtrate using buffers ranging from pH 5–9.

- **Synthesis by bacteria**

Beveridge and Murray conducted their first research on the biosynthesis of gold nanoparticles (GNPs) using the bacteria *Bacillus subtilis* in 1980. Since then, a variety of microorganisms have been used to synthesize a variety of metals, nonmetals, metal oxides, and bimetallic nanoparticles, with more applications being considered. The use of marine bacteria to synthesize gold and silver nanoparticles has been active in recent years, as has the novel bacterial strain *Marinobacteralgicola,* which was isolated from marine waters in the Indian Sector's Southern Ocean. Furthermore, several bacteria, including strains of *Bacillus*, *Cupriavidus*, and *Shewanella*, were discovered to be capable of reducing Au(iii) to Au NPs (Liu et al., 2018). A method that involves isolating the bacterium from water samples and growing it in broth for 24 h. Centrifugation is a method of harvesting biomass. Cell biomass is used to determine whether the enzyme responsible for GNP preparation is intracellular or extracellular. The cell biomass was washed twice in a phosphate buffer (pH 7, 0.05 M), then dissolved in 50 ml of distilled water and ultrasonicated (5 min, 30-second pulse) to break down the cell wall and release the enzyme into the aqueous system. The pellet is discarded by centrifugal solution and cell lysate supernatant (CLS). In a flask, ($HAuCl_4$–1 mM) was mixed with (25 ml of supernatant) and stirred at 30 °C at 150 rpm for 72 h to produce AuNPs. Following an ultrasound, there was a notable shift of color from cell biomass. This shows that the enzyme is involved in intracellular processes in nature *(Gupta and Padmanabhan, 2021)*

- **Synthesis by plant**

Although chemical methods are the most common approach for the synthesis of metallic nanoparticles, the use of expensive and toxic reagents as reducing and stabilizing agents limits their applications. In addition, these nanoparticles may have harmful effects in biomedical applications. Hence, there is a growing need to develop eco-friendly and cost-effective procedures for the synthesis of nanoparticles that do not use any toxic chemicals. 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 (Singh et al. 2013). 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. In recent years, the biosynthesis of AuNPs using plants such as *Azadirachta indica, Cannabis sativa, Medicago sativa, Aloe vera, Cinnamomum camphora, Pelargonium graveolens, Coriandrum sativum, Terminalia catappa, and lemongrass* have been reported. Plants of the genus *Cannabis* are found in the northern hemisphere and produce more than 400 known secondary metabolites; more than 60 of which are cannabinoid compounds.*Cannabis sativa* is an annual herbaceous medicinal plant in the *Cannabis* genus, a species of the Cannabaceae family. The genus has two main subspecies: *C. sativa and Cannabis indica*(Pearce et al., 2014) *. C. sativa* is the tallest one with pale green thin leaves, and it is one of the fastest growing plants. It is very popular for industrial use due to its low but variable lignin content and enrichment with bast fibers. This makes the plant's fibers applicable for producing textiles, paper, ropes, biofuel, biodegradable plastics, insulation, paint and animal feed. *C. sativa* plants have a higher content of psychoactive tetrahydrocannabinol (THC) compared with *C. indica*. During the past few decades, the plant has been increasingly used for medicinal treatments against various diseases such as inflammation, cancer, obesity, osteoporosis, multiple sclerosis, emesis, epilepsy, pain, glaucoma, anorexia, etc.(*Bar-Sela et al., 2013).* *Cannabis sativa* (hemp) is a source of various biologically active compounds, for instance, cannabinoids, terpenes and phenolic compounds, which exhibit antibacterial, antifungal, anti-inflammatory and anticancer properties. With the purpose of expanding the auxiliary application of *C. sativa* in the field of bio-nanotechnology,explored the plant for green and efficient synthesis of gold nanoparticles (AuNPs).

Characterization of nanoparticles

Uv visible spectroscopy

UV–Visible spectroscopy is generally used to confirm the synthesis and stability of metal NPs/colloidal particles. Synthesis is confirmed based on the absorbance of the samples at wavelength from 230 to 800 nm, and NPs ranging from 1 to 100 nm can be used for

analysis. UV absorption spectroscopy is not only used for the confirmation of synthesis, but also used for the quantitative determination of particles in colloidal solutions. Furthermore, absorption spectra determine the size of the particles and can be used for quantitative and qualitative analysis of particles. Spectroscopy involves the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules, atoms, or ions of a sample move from one energy state to another. Nanoparticles samples are analyzed using the absorption of ultraviolet light (200–400 nm) by the molecule which results in the excitation of the electrons from the ground state to higher energy state. The salient features of UV–Vis spectroscopy are easy handling, sealed optics, double choppers, and the spectral bandwidth, which can be set to 0.2 nm.*(Kadziola et al., 2013)*

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) is considered a powerful and simple technique. It has an imperative role in biological systems for measuring the concentration of chemicals, surface chemistry, functional group, and atomic arrangement of the biological nanoparticles samples *(Cao, 2004).* In nanoparticles synthesis, FTIR can analyze whether the biomolecules are involved in synthesis or not. It also shows what biomolecules are present in the sample *(Perevedentseva et al., 2010).* FTIR measurement depends on the vibration of molecular bonds positioned at various frequencies and the type of bonds. The salient features of FTIR are high sensitivity, high cube corner interferometer, customizable workspaces, and hyperspectral imaging.

Dynamic light scattering

Dynamic light scattering (DLS), also called photon correlation spectroscopy and elastic light scattering *(Dzakpasu & Axelrod, 2004),* involves a table top instrument and is an easy-to-handle technique. Furthermore, the method is accurate, less time consuming, inexpensive, and data reproducible and allows analysis of high-molecular-weight samples. It is mainly used to evaluate the size and surface charge of NPs. Colloidal dispersed NPs can only be measured by Brownian motion *(渡辺知規 et al., 2004)*. It also has certain limitations such as aggregation and is not suitable for the analysis of non-spherical nanomaterials with heterogeneous size distributions. The working principle of

the DLS involves screening the elastic scattering intensity of light from the Brownian motion of the sample. The particle size can be obtained from the motion-dependent auto correlation function of Einstein equation *(DeLano, 2009)*. The salient features of DLS include measurement of samples ranging in size from 0.3 nm to 6 µm and a minimum volume 10 µL of sample suspension with an accuracy of ±2% and a precision of ±1%. It can measure the zeta potential of colloidal, nanoparticulate, and macromolecular samples in the size range of 1 nm to 100 µm with a minimum volume of 175 µL.

Electrophoretic light scattering

Electrophoretic light scattering (ELS) is a technique used to measure the electrophoretic mobility of particles in dispersion, or molecules in solution. This mobility is often converted to Zeta potential to enable comparison of materials under different experimental conditions. The basic principle of this instrument is electrophoresis, which is mainly based on electric charges, when electric field is applied into the dispersion, particles, or molecules are having net zeta potential will migrate towards the oppositely charged electrode with a velocity, known as the mobility, that is related to their zeta potential. Malvern Instruments offers equipment to measure the electrophoretic mobility of particles using electrophoretic light scattering. For example, The Zetasizer Nano provides a simple, fast, easy, and accurate way to measure zeta potential and free from cross contamination due to use of unique disposable capillary cell.

X-Ray diffrction

XRD is one of the best methods for characterization of the crystalline form of organic and inorganic materials.XRD is nondestructive, simple, highly sensitive, depth profile, table make, and user friendly. It also contains several application aspects such as pharmaceutical, glass, polymer, geological, and forensic and has been used to analyze the chemical composition by quantitative and qualitative measurements by measuring the degree of crystallinity and providing accurate information on the atomic arrangements at interfaces. The crystal structure describes the atomic arrangement, position, and intensity of the diffraction peaks. The wavelength of X-rays is on the atomic scale. Therefore, it is mainly used for probing the structure of nanomaterials. A single beam of X-rays is scattered by each atom in the powder sample. The scattered beams reflected by any

crystal form many diffraction patterns. Where the X-rays scattered sample the maximum intensity of the peak at a particular angle. This peak reflects the structural and physicochemicalcharacters of the crystal. The working principle of XRD follows Bragg's Law *(Waseda et al., 2011).*

Scanning electron microscopy

SEM is a versatile, nondestructive analytical method that involves a microscope with a large specimen chamber, with a working distance of 8.5 mm, owing to a combination of inclined detectors and the sharp conical objective lens. SEM is used for surface and dimensional measurements of nano and micro structure analysis of samples and is a type of imaging technique. It is a valuable tool for the evaluation of material structure. It is fast and easy to operate and provides reliable data. In addition, SEM is applied in numerous fields such as biological science, biomolecules, biomedical fields, and material sciences. The working principle of SEM is based on generation of electron beams that have magnetic properties. Their magnetic field interacts with the sample to produce secondary and backscattered electrons, which are used for detection *(Hawkes, 2015).* The detection of transmitted electrons is very useful to study nanomaterials. Furthermore, the elemental composition and concentration of samples can be analyzed using SEM-EDX

Transmission electron microscopy

TEM is a common method and an indispensable tool for the characterization of NPs. This main advantage is the determination of the morphology, crystal structure, and size and the qualitative and quantitative analysis of prepared NPs and internalized NPs in cells or tissues (*Pulskamp et al., 2007)*. Conventional TEM techniques are used to analyze the sample ubiquitously. However, this technique has certain limitations related to samples >300 nm thick, only limited areas of which are screened; for particle size >100 nm, low magnification is achieved; TEM cannot characterize small-sized NPs (10–20 nm or less than 10 nm); and sample preparation is destructive. TEM is classified based on applications like immune and energy filtered electron microscopy.

Anticancer activity of AuNPs

Over the past decade, the investigation of inorganic nanoparticles for biomedical application becomes a fast-growing area of research with great fascination. Distinct physicochemical properties of AuNPs make them ideal for biomedical applications. Green synthesis of AuNPs utilizes medicinally important plant extracts, which may remain on the surface of nanoparticles and, in such condition, AuNPs act as carriers.The cytotoxic activity of green synthesized AuNPs against different cancer cells. The use of AuNPs also minimizes the risk of side effects and limits the damage to normal (noncancerous) cells *(Tiloke et al. 2016)*. AuNPs are a novel agent in cancer therapy and size-dependent cytotoxic activity against different cancer cells *(Cui et al. 2012)* which also depends on the dose of nanoparticles. AuNPs are receiving significant attention from researchers because of their biocompatibility and unique property to conjugate with proteins *(Fang et al. 2010).* The mechanism behind the anticancer activity of AuNP is quite complicated and not well understood. AuNPs are considered as a carrier for phytocomponents and may act as an anticancer agent. The mechanism behind its activity is only provisionally described. The interaction between AuNPs and cells differs in numerous ways; many researchers have reported the cellular internalization of AuNPs *(Gong et al. 2015).*The surface properties of AuNPs are most important factor in internalization by cells. AuNPs carry positive charges, while cancer/normal cell membranes contain negatively charged materials like lipids (especially phosphate groups); having opposite charges is responsible for AuNPs uptake and internalization.Another way for the entry of gold nanoparticles into cells is endocytosis, a study wherein tiny AuNPs were endocytosed and showed aggregation inside HeLa cells. The AuNPs showed cytotoxic activity via ROS production and activation caspase cascade of apoptosis and mitochondrial dysfunctioning *(Tiloke et al. 2016).*

OBJECTIVE

Objectives

- Biological synthesis of gold nanoparticles by using *Cannabis sativa* leaf extract.
- Characterization of gold nanoparticles by UV-Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential.
- Assessment of the potency of gold nanoparticles against cervical cancer HeLa cells.

MATERIAL AND METHOD

Material and methods

Chemical

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

Plant Collection and extract preparation

Cannabis sativa plant was kindly gifted from Dr. Maqbool Ahmad Khan Deputy Director of CCRMU, Kursi Road, Basaha, 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.

Cannabis sativa 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 48hrs 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 were 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 analyzer (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 CNB-extract CNB-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.

Measurement of morphological changes in HeLa cells

HeLa cells were pre-treated with different concentrations of each, CNB- Extract and CNB-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).

Assessment of cytotoxicity

To assess the cytotoxic effect of CNB-extract and CNB-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 CNB-extract and CNB-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:

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

RESULT AND DISSCUSSION

Result and discussion

Cannabis sativa-Mediated synthesis of AuNPs (CNB-AuNPs)

This study used *Cannabis sativa* leaf extract as a reducing and capping agent, whereas 1Mm gold chloride ($HAuCl_4$) served as the gold precursor. The synthesis of CNB-AuNPs is considered to be induced by the aqueous extracts reducing enzymes and capping agents, such as secondary metabolites. The creation of CNB-AuNPs was confirmed visually by a shift in the color of the extract from green to ruby red, indicating gold reduction.

Characterization of CNB-AuNPs

The phyto-constituents in *Cannabis sativa* 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 CNB-AuNPs. The change in color from light green to ruby red indicated the successful synthesis of CNB-AuNPs and the result of SPR (Surface Plasma Resonance) band confirm that at 530 and however there was no discernible peek for cannabis sativa 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 CNB-AuNPs had an average particle size of 58.46 d nm as shown in figure. Furthermore, the Zeta potential of the prepared CNB-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.

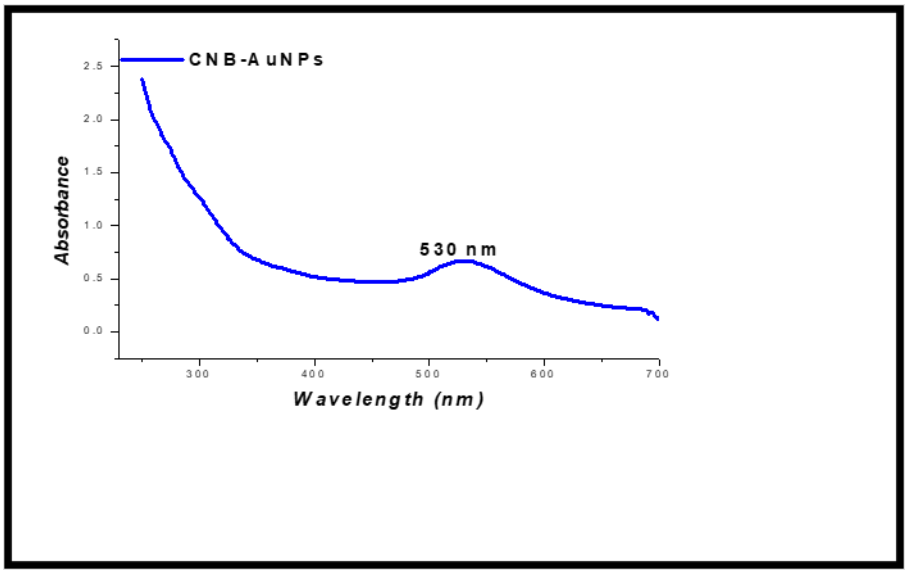

Figure-3: Characterisation of CNB-AuNPs under UV-Visible spectra (530 nm).

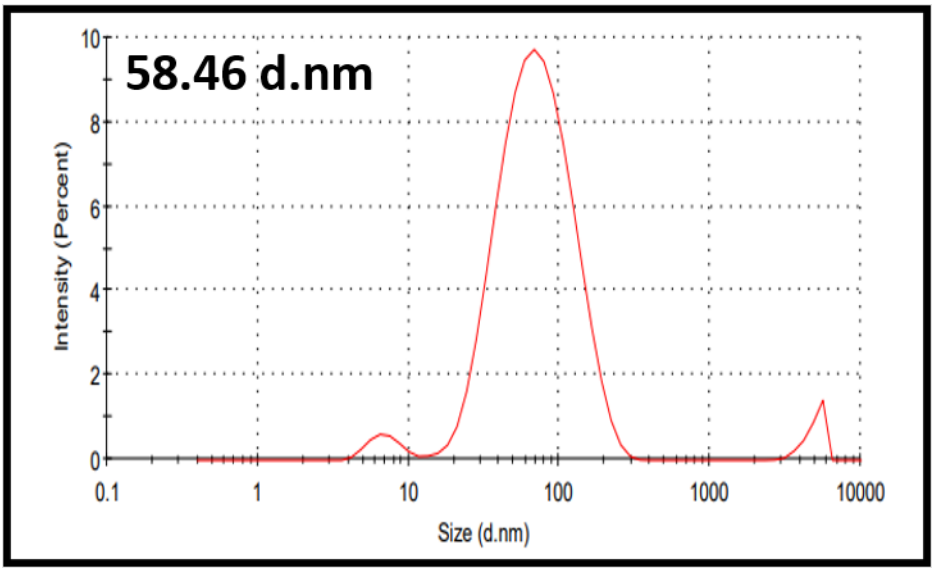

Figure-4: DLS profile of CNB-AuNPs showing size of 58.46 d.nm

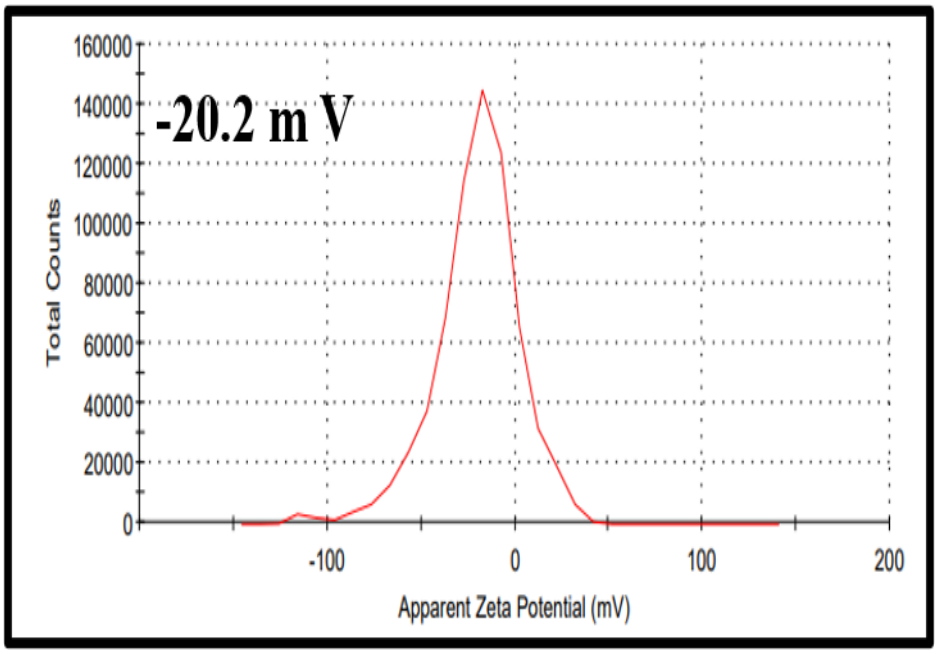

Figure-5: Zeta potential of CNB-AuNPs confirmed the stability at -20.2 mV.

Determination of morphological alterations in Cervical cancer cells

Morphological analysis of the CNB-extract and CNB-AuNPs, treated cervical cancer cells was performed using a phase contrast microscope. A dose dependent change in the cell morphology was observed in HeLa cells after treatment with CNB-extract (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml) and CNB-AuNPs (20 µg/ml, 40µg/ml, 80µg/ml) concentrations for 24 h. In the presence of different doses CNB- extract and CNB-AuNPs, Cervical cancer 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 cells. These morphological changes in cervical cancer cells were more evident with the increase in the dose in CNB-AuNPs. In contrast, well spread flattened morphology was observed in untreated control cells.

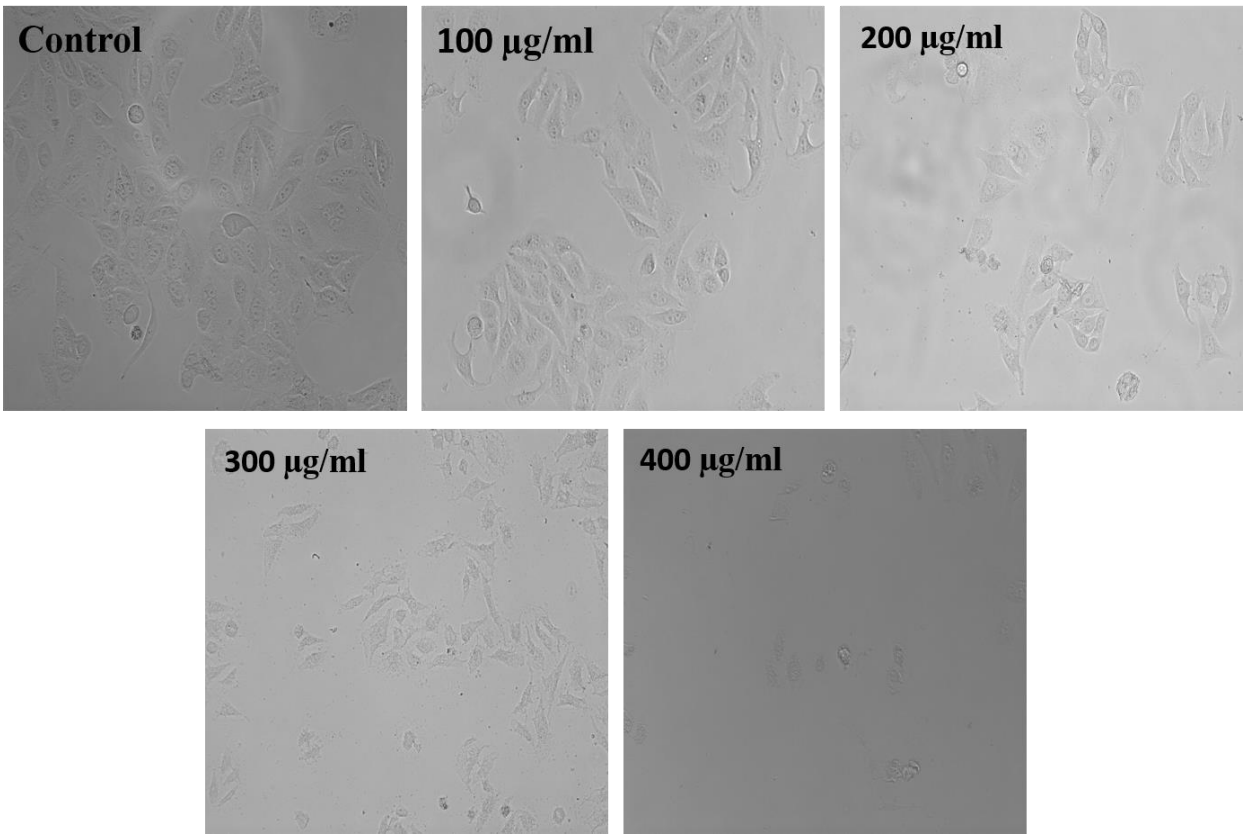

Figure-6: The phase contrast microscopy of cervical cancer cells treated with either vehicle control or different concentration (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml) of CNB-extract for 24hours in a time and dose- dependent manner. Images shown are representative of three independent experiments (Scale bar:100µm; Magnification: 20X)

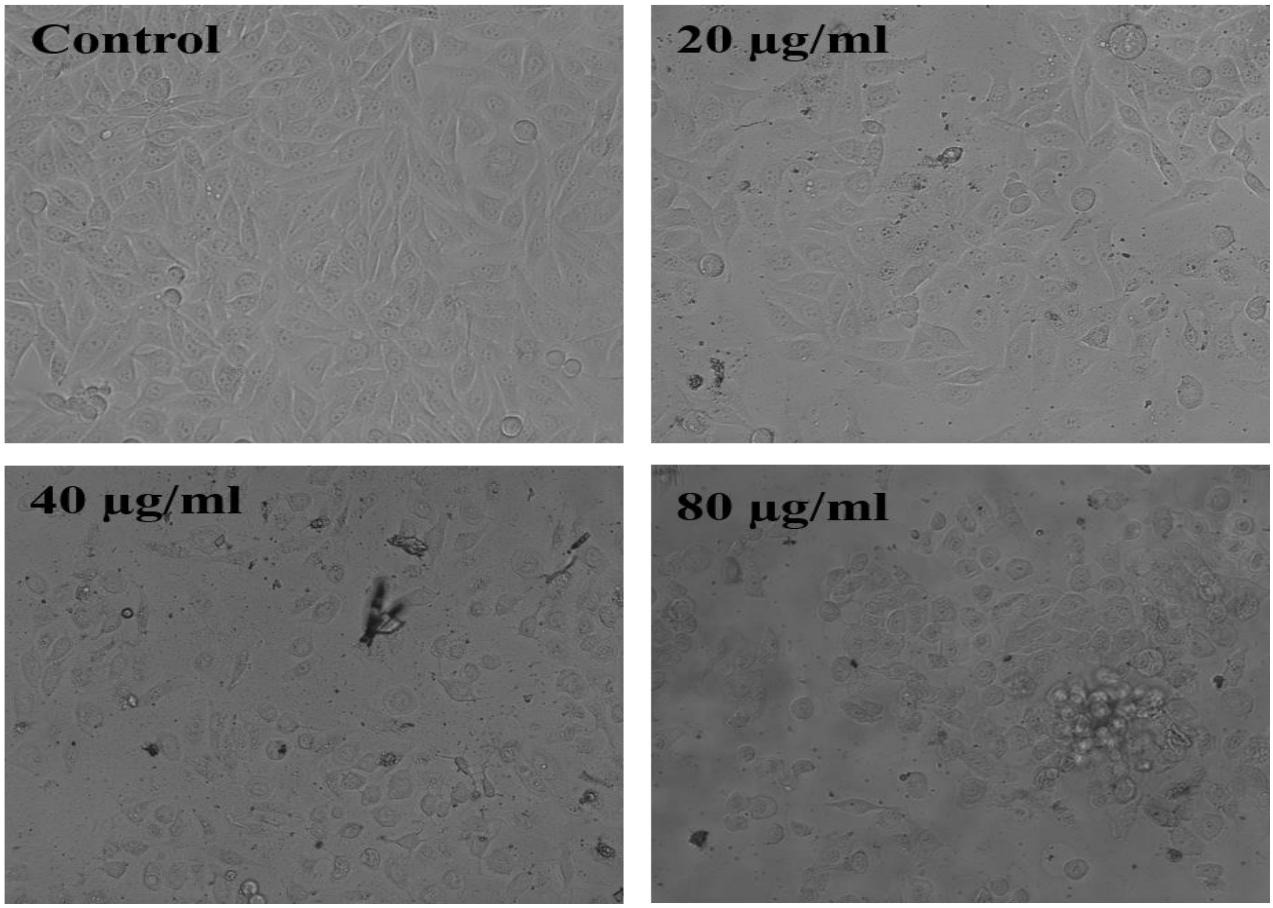

Figure-7: -The phase contrast microscopy of cervical cancer cells treated with either vehicle control or different concentration (20 µg/ml, 40 µg/ml&80 µg/ml) of CNB-AuNPs for 24hours in a time and dose- dependent manner. Images are shown are representative of three independent experiments (Scale bar:100µm; Magnification:20X)

In vitro cytotoxicity of CNB-extract and CNB-AuNPs

To evaluate the sensitivity of cervical cancer cells to these drugs, cervical cancer cells were treated with different doses of CNB- extract and CNB-AuNPs, at a concentration of 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 20 µg/ml, 40 µg/ml, 80 µg/ml respectively for 24 h followed by MTT assay. Our results showed that, after 24 h of treatment, CNB-extract at IC50 concentration exhibited 152.4±1.12µg/ml reduced growth of cervical cancer cells by 50%, while inhibition of 50% exhibited IC50 concentration of 24.38±1.13µg/ml, CNB-AuNPs, respectively. CNB-AuNPs, were found to be more

cytotoxic for cervicalcancer cells in comparison to pure extract and the effect was observed to be dose-and time-dependent.

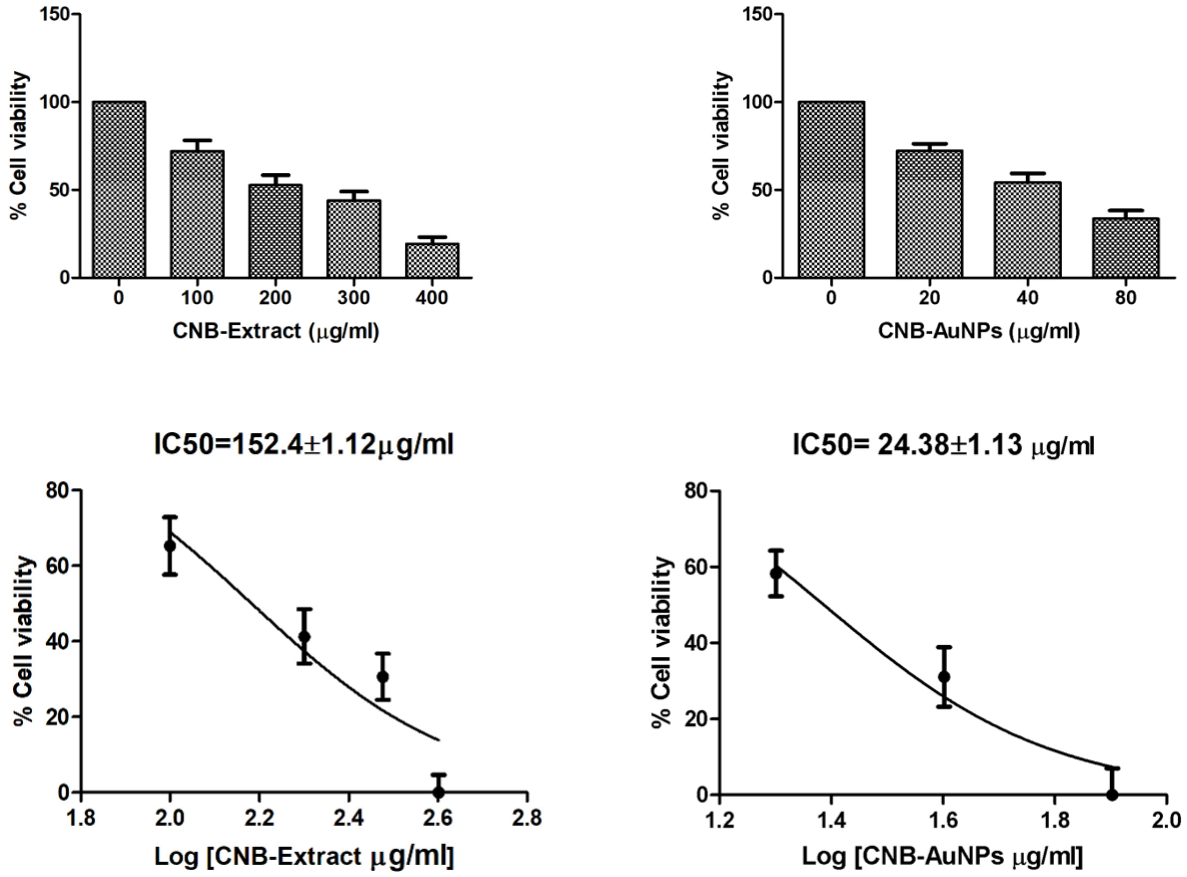

Figure-8: Percent cell viability of cervical cancer cell with different doses of *Cannabis sativa* (100-400µg/ml) assessed by MTT Assay 24 h. Graph showed that *Cannabis sativa* exhibited IC50 value 152.4±1.12 µg/ml and 24.38±1.13µg/ml at 24 h, against cervical cancer cells. The result represented are the mean ±SEM of three independent experiment performed in triplicate.

CONCLUSION

Conclusion

In this study we showed a leaf extract mediated green synthesis of gold nanoparticles from *Cannabis sativa* plant and their characterization, anticancer and antioxidant property analysis. This study investigates an efficient and sustainable route of AuNPs preparation from 1mM aqueous $AuCl_4$ using leaf extracts of *Cannabis sativa* 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 anticancer and antioxidant activity and also 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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