A DISSERTATION ON

"BIOGENIC SYNTHESIS OF GOLD NANOPARTICLES BY USING *WITHANIA SOMNIFERA* LEAF EXTRACT AND EVALUATION OF THEIR CYTOTOXIC EFFECT ON CERVICAL CANCER HeLa CELLS"

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FOR THE
DEGREE OF MASTER OF SCIENCE
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BY

FAISAL KHAN
M.Sc. Biotechnology (IV Semester)
Department of Biosciences
INTEGRAL UNIVERSITY, LUCKNOW

UNDER THE SUPERVISION OF

Dr. IRFAN AHMAD ANSARI
Associate Professor
Department of Biosciences
Integral University, Lucknow

INTEGRAL UNIVERSITY

Established Under U.P. Act No 09 of 2004 by State Legislation
Approved by University Grants Commission
Phone No.: +91 (0552) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026, Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Mr. FAISAL KHAN**, student of M.Sc. Biotechnology (II Year, IV semester), Integral University has completed his four months dissertation work entitled ***"Biogenic synthesis of gold nanoparticles by using Withania somnifera leaf extract and evaluation of their cytotoxic effect on cervical cancer HeLa cells"*** successfully. He 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 his M.Sc. degree. I wish him good luck for the future endeavours.

Dr. Snober S. Mir
Head,
Department of Biosciences,
Integral University, Lucknow

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TO WHOM IT MAY CONCERN

This is to certify that the study conducted by **Mr. FAISAL KHAN** 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 himself. The thesis entitled is ***"Biogenic synthesis of gold nanoparticles by using Withania somnifera leaf extract and evaluation of their cytotoxic effect on 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

Dr. Irfan Ahmad Ansari

(Supervisor)

Associate Professor
Department of Biosciences
Integral University, Lucknow

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Date: ***FAISAL KHAN***

CONTENTS

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
nm	Nano meter
MIC	Minimum Inhibitory Concentration
AuNPs	Gold nanoparticles
NPs	Nanoparticles
WS	*Withania somnifera*

INTRODUCTION

Introduction

The word "nano" is taken from the Greek word "nanos" and that implies little and it is utilized as the prefix for one billionth fraction (10^{-9}) (Khan *et al.,* 2014). As per American Society for Testing and Materials nanoparticles are those particles which have at least two or more aspects and are in the size scope of 1 - 100 nm (Alanazi *et al.,* 2010). These particles have unique and upgraded physiochemical properties than their mass materials because of their enormous responsive and uncovered surface region and quantum size impact because of explicit electronic designs. These particles have been generally utilized in many fields like gadgets, photochemical, biomedicine and chemical sciences (di Guglielmo *et al.,* 2010).

Nanoparticles are being utilized for various purposes, from clinical medicines, utilizing in different parts of industry creation, for example, sun powered and oxide fuel batteries for energy conservation, to wide consolidation into different materials of daily use like beauty care products or garments (Dubchak *et al.,* 2010). Nanoparticles which are produced nowadays are copper, zinc, titanium, magnesium, gold, alginate and silver nanoparticles. The field of nanotechnology is growing rapidly, in which nanoparticles are being used in various medical diagnosis and treatment, due to their little sizes, high surface-to-volume proportion and special optical characteristics *(*Bogart *et al.,* 2014; Chen *et al.,* 2016). As an example, GNPs show various applications in the identification of small analytes and biomolecules through surface alteration (Yuan *et al.,* 2016).

Gold Nanoparticles (AuNPs) are nanoparticles which are produced using gold salt which is AuCl4. Properties of gold nanoparticles are not quite the same as its mass structure since mass gold is yellow strong and it is dormant in nature while gold nanoparticles are wine red solution and are accounted for to be against oxidant (Khan *et al.,* 2014). Inter molecule cooperation and joining of gold nanoparticles connection perform key part in the assurance of characteristics of these nanoparticles (Deb *et al.,* 2011). AuNPs ranges from 1-8 micrometre and are of different shapes characterized as Nanosphere, Nanocluster, Nanorod, Nanostar and Nanocubes etc. GNPs have been broadly utilized in the field of radiation treatment as radiation enhancer and furthermore give remedial improvement in radiation treatment due to the effective and targeted on drug delivery to the cancer site (Ganeshkumar *et al.,* 2012). Gold

nanorods certainly got more attention in previous years due to their particular optical and chemical characteristics and consequently utilized for their applications in biological sciences (Pal *et al.,* 2013). Gold nanoparticles have various applications as platform nanomaterials for biomolecular ultrasensitive detection, killing cancer cells by hyperthermal treatment, labelling for cells and proteins and delivering therapeutic agents within cells (Khan *et al.,* 2014).

Withania somnifera (L.) is a miracle herb with various therapeutic properties and its root is utilized in the production of numerous Ayurvedic medicines. Withanolides, steroidal lactones, present in the root are functional chemical markers, nevertheless, phenolics, and flavonoids have likewise been accounted for in the root of this plant (Dhanani *et al.,* 2017). Withania somnifera ordinarily known as Ashwagandha, is a green bush found all through the drier lands of Asia, South Africa, and Egypt (Kulkarni *et al.,* 2008). In India, it is grown broadly in north west and central India (Bhatia *et al.,* 1987). *Withania somnifera* shows significant anti tumor activity against prostate cancer where the utilization of *W. somnifera* came about in metabolic inactivation Cdc2 catastrophe followed by cell death, obviously exhibiting the capability of *W. somnifera* as a regulator of G2/M phase cycle of cancer cells and its activity against prostate cancer. (Roy *et al.,* 2013). The binding particles of the *W. somnifera* alkaloid at the site of the protein known as BIR5 helps interruption of the mitosis and furthermore shows their capability as an antitumor drug (Wadegaonkar *et al.,* 2013). *W. somnifera* might be utilized as an adjuvant during chemotherapy for counteraction of bone marrow melancholy connected with anticancer medications (Gupta *et al.,* 2001).

In 2014, Bindhani et al., demonstrated the synthesis of gold nanoparticles using leaf extract of *Withania somnifera,* and it appeared to be eco-friendly. This method could be used for fast and easy synthesis of gold nanoparticles. The size of the nanoparticles can be without any problem changed by utilizing various measures of leaf extract (Bindhani *et al.,* 2014) .

Cancer is described by an over-multiplication of diseased cells with high limit with respect to replication and invasion (Rai *et al.,* 2016). Metastasis, the spread of cancer cells from a primary organ to other organs, represents the most serious issue to tumor medication and is the primary reason for death cancer patients(Geiger *et al.,* 2009).

Metastatic cells are frequently exceptionally dangerous, hard to locate & distinguish, and chemoresistant *(*Gunasinghe *et al.,* 2012).

Cervical cancer is the fourth general type of cancer in females throughout the world and addresses a significant worldwide health problem. In 2018, an expected 569847 new patients of cervical malignant malignancy were reported and 311 365 deaths happened around the world because of this cancer (Bray *et al.,* 2018). For a long time, cervical cancer has been perceived as a disease which is transmitted during sexual intercourse. There is also proof that HVP is a sexually transmitted infection whereas fomite transmission appears to be quite possible because HPV DNA has been found from undergarments and gynaecologic materials (Franco *et al.,* 1991*;* Zur *et al.,* 1994). The therapy of cervical cancer is specifically based on stage of cancer. While beginning phase infection can be treated with radiotherapy or surgical procedure, the best therapy for advanced stage patients is simultaneous chemotherapy and pelvic irradiation (Kang *et al.,* 2015*;* Lorusso *et al.,* 2014). Biologically synthesized gold nanoparticles are used in treatment of cervical cancer by inducing apoptosis in diseased cells *(*Ke *et al.,* 2019*;* Qian *et al.,* 2019).

AuNPs are utilized in cancer therapy, because of their particular properties, such as their capability of interaction with various medicines, control in cancer tissues, infrared light absorbance and their correspondence with radiations (Hussain *et al.,* 2021). AuNPs especially have significantly centred around for cancer research in earlier years because of their easy synthesis as well as surface varieties, enormously improved and alterable optical properties including remarkable biocompatibility for clinical methodologies (Gao *et al.,* 2012).

REVIEW OF LITERATURE

Review of Literature

Nanobiotechnology

Nanobiotechnology is the application of nanotechnologies in biological fields. Chemists, physicists, and biologists each view nanotechnology as a branch of their subject, and collaborations in which they each contribute equally are common. One result is the hybrid field of nanobiotechnology that uses biological starting materials, biological design principles, or has biological or medical applications. While biotechnology deals with metabolic and other physiological processes of biological subjects including microorganisms, in combination with nanotechnology, nanobiotechnology can play a vital role in developing and implementing many useful tools in the study of life. Although the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, therapy, and drug delivery vehicles, bionanotechnology research is still in its infancy (Chritof *et al.,* 2004). The prefix 'nano' is referred to as a Greek prefix meaning 'dwarf' or something very small and depicts one thousand millionths 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 (Dean *et al.,* 2005). As a comparison, one must realize that a single human hair is 60,000 nm thickness and the DNA double helix has a radius of 1 nm (Gnach *et al.,* 2015). The development of nanoscience can be traced to the time of the Greeks and Democritus in the 5th century B.C. when scientists considered the question of whether the matter is continuous, and thus infinitely divisible into smaller pieces, or composed of small, indivisible and indestructible particles, which scientists now call atoms. 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. 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" (An official website of the United States government, n.d.). This definition suggests the presence of two conditions for

nanotechnology. The first is an issue of scale: nanotechnology is concerned to use structures by controlling their shape and size at the nanometre scale. The second issue has to do with a novelty: nanotechnology must deal with small things in a way that takes advantage of some properties because of the nanoscale (Allhoff *et al.,* 2007). We should distinguish between nanoscience and nanotechnology. Nanoscience is a convergence of physics, materials science, and biology, which deal with the manipulation of materials at atomic and molecular scales; while nanotechnology is the ability to observe measure, manipulate, assemble, control, and manufacture matter at the nanometre scale. There are some reports available, which provided the history of nanoscience and technology, but no report is available which summarize the nanoscience and technology from the beginning to that era with progressive events. Therefore, it is of the utmost requirements to summarize main events in nanoscience and technology to completely understand their development in this field (*Bayda et al., 2019*). After the discovery of this new field of research catches the interest of many scientists, two approaches have been developed describing the different possibilities for the synthesis of nanostructures. These manufacturing approaches fall under two categories: top-down and bottom-up, which differ in degrees of quality, speed, and cost. The top-down approach is essentially breaking down of bulk materials to get nano-sized particles. This can be achieved by using advanced techniques such as precision engineering and lithography which have been developed and optimized by industry during recent decades. Precision engineering supports the majority of the micro-electronics industry during the entire production process, and the high performance can be achieved through the use of a combination of improvements. These include the use of advanced nanostructure based on the diamond or cubic boron nitride and sensors for size control, combined with numerical control and advanced servo-drive technologies. Lithography involves the patterning of a surface through exposure to light, ions or electrons, and the deposition of material on to that surface to produce the desired material. The bottom-up approach refers to the build-up of nanostructures from the bottom: atom-by-atom or molecule-by-molecule by physical and chemical methods that are in a nanoscale range (1 nm to 100 nm) using controlled manipulation of self-assembly of atoms and molecules. Chemical synthesis is a method of producing rough materials which can be used either directly in the product in their bulk disordered form, or as the building blocks of more advanced ordered materials. Self-assembly is a bottom-up approach in which atoms or

molecules organize themselves into ordered nanostructures by chemical-physical interactions between them. Positional assembly is the only technique in which single atoms, molecules, or clusters can be positioned freely one-by-one (Iqbal *et al.,* 2012).

Nanoparticles

Nanoparticles are considered a discovery of the twentieth century, but a brief overview of the field reveals that artisans in Mesopotamia used finely divided materials of this type as early as the ninth century BC, to obtain a glittering effect on the surface of ceramic vessels. In the Middle Ages and the Renaissance, the production of glittering metallic films led to methods of covering glassy surfaces developed in various Far Eastern or European centres which became famous thanks to these methods that are largely employed even today. During the Renaissance and in later times, the development of visual arts (painting in particular) and printing and engraving methods contributed to the technique of producing fine inorganic and organic dust particles, close to nanoparticles in size, and dispersions that were stable in different solvents and used as dyes or ink *(*Radu *et al.,* 2004). Michael Faraday was the first to provide a scientific description of the optical properties of nanometric metal particles, in a paper he published in 1857 (Faraday *et al.,* 1857). Turner further revealed that leaving very thin gold or silver films on glassy surfaces heated at 500ºC changed both the properties of the deposited metals and those of the glass so that white light crossed the metallic film; this caused a marked reduction of reflection, while electrical resistivity increased significantly (Turner *et al.,* 1908). Ten years later (1867), James Clark Maxwell suggested a series of concepts of differentiation in nanotechnology, but without using the word “nanotechnology” to define thin, monomolecular layers. The shortest definition of nanoparticles, which is probably the most intuitive one, takes into consideration only their size, which is limited conventionally to about 100 nm in any direction. This definition cannot be exhaustive, as it does not give net values. But without a classification, no matter how general, it is difficult to differentiate between the molecular and atomic field on the one hand and the nanoparticle field on the other. Nanoparticles can be classified based on the following criteria (Braga *et al.,* 1994*;* Klabunde *et al.,* 2012) :

- Origin
- Natural

- Anthropogenic
- Size:
  i. 1-10 nm
  ii. 10-100 nm
  iii. Over 100 nm
- Chemical composition:
  1. Inorganic substances
  2. Organic substances
  3. Elements of the living kingdom.

Nanoparticles are of great scientific interest, as they are a bridge between bulk substances and their molecular or atomic structure. A bulk substance has constant physical and chemical properties irrespective of its size; nevertheless, at the nanoscale, these properties depend on more or less discreet molecular or atomic phenomena. Regardless of the nature of nanoparticles, their most important physical properties are the following:

1. **Surface area:** It has been found that properties vary with particle size. In sub-micrometre particles, forces that govern the atomic or molecular universe dominate to the detriment of statistical aspects, which are revealed at the macroscale.
2. **Optical properties:** Nanoparticles often have particular optical properties, as they are small enough to limit the thickness of the common electron layer of metals; this phenomenon generates quantum effects. Although it is common knowledge that gold is yellow and silicon is grey, gold, and silicon nanoparticles are bright red to black. Moreover, gold nanoparticles melt at a much lower temperature (~ 300ºC, 2.5 nm size) than gold slabs that melt at 1064ºC. Solar energy absorption in photovoltaic cells made of silicon-based nanomaterials is much higher than in thin films of the same materials. The smaller the particles, the higher the absorption efficiency.
3. **Uniformity:** Clusters, aggregates, or filaments, in other words, the molecular or atomic assemblies that form nanoparticles, are defined by the interaction of forces among the molecules or atoms of a particle and the interaction forces among particles.
4. **Functionalization:** Nanoparticles of any type can be linked to microbiological entities randomly, through natural processes occurring in the atmosphere, water or

at the surface of the Earth. Nanoparticles are then directed to living organisms, organelles within the cells, and individual protein or RNA molecules. This property is related both to the harmful effects of nanoparticles on the living kingdom and the pharmaceutical or biochemical studies conducted voluntarily, to the level of peptide molecules.

5. **Quantum confinement:** Changes in size-dependent properties also include quantum confinement, a phenomenon that causes spontaneous properties of semi conductivity, conductivity, or electric insulation for neighbouring particles less than 10 nm in diameter (Strambeanu *et al.,* 2015).

Metal Nanoparticles

Metallic nanoparticles or metal nanoparticles, a new terminology has been originated in the field of nanoparticles in recent few years. The noble metal like gold, silver, and platinum having beneficial effects on health are utilized for the synthesis of nanoparticles and designated as metallic nanoparticles *(*Bhattacharya *et al.,* 2008). Nowadays researchers are focusing on metal nanoparticles, nanostructures, and nanomaterial synthesis because of their conspicuous properties that are useful for catalysis (Narayanan *et al.,* 2004), composite like polymer preparations (*Moura et al., 2017*), disease diagnosis and treatment (Banerjee *et al.,* 2017), sensor technology (Banerjee *et al.,* 2017*;* Gomez *et al.,* 2001), and labelling of optoelectronic recorded media (Shaikh *et al.,* 2016). Metallic nanoparticles have a specialty with appropriate functional groups. It can be synthesized and modified that would allow them to bind with ligands, antibodies, drugs (Prasad *et al.,* 2013). Metallic nanoparticle is nanosized metals with a size range of 10- 100nm. Metallic nanoparticles have unique characteristics such as surface Plasmon resonance and optical properties (Kumar *et al.,* 2018).

Gold Nanoparticles (GNPs)

Gold nanoparticles (GNPs) 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 oligonucleotides, (Guo *et al.,* 2010*;* Park *et al,* 2010) antibodies,(Eck *et al.,* 2008*;* El-Sayed *et al.,* 2005) , 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 (Moyano *et al.,* 2011). Spherical AuNPs possess

useful attributes such as size- and shape-related optoelectronic properties, (Sau *et al.,* 2010) large surface-to-volume ratio, excellent biocompatibility, and low toxicity *(*Khlebtso *et al.,* 2011*)*. These properties make AuNPs an important tool in bionanotechnology. Important physical properties of AuNPs include surface plasmon resonance (SPR) and the ability to quench fluorescence. Spherical AuNPs exhibit a range of colours (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 *(*Eustis *et al.,* 2006*;* Jain *et al.,* 2006).

Applications of GNPs

The range of applications for gold nanoparticles is growing rapidly and includes:

i. **Electronics -** Gold nanoparticles are designed for use as conductors from printable inks to electronic chips (Huang *et al.,* 2003). As the world of electronics become smaller, nanoparticles are important components in chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip.

ii. **Photodynamic Therapy -** Near-IR absorbing gold nanoparticles (including gold nanoshells and nanoparticles) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these nanoparticles to eradicate targeted tumors (Stuchinskaya *et al.,* 2011). When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.

iii. **Therapeutic Agent Delivery -** Therapeutic agents can also be coated onto the surface of gold nanoparticles (Brown *et al.,* 2010). The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers).

iv. **Sensors -** Gold nanoparticles are used in a variety of sensors. For example, a colorimetric sensor based on gold nanoparticles can identify if foods are suitable for consumption (Ali *et al.,* 2012). Other methods, such as surface-enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds. This

strategy could also be used for the detection of proteins, pollutants, and other molecules label-free.

v. **Probes -** Gold nanoparticles also scatter light and can produce an array of interesting colours under dark-field microscopy. The scattered colours of gold nanoparticles are currently used for biological imaging applications (Perrault *&* Chan*,* 2010). Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.

vi. **Diagnostics -** Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agent (Peng *et al.,* 2009). They are also common in lateral flow immunoassays, a common household example being the home pregnancy test.

vii. **Catalysis-** Gold nanoparticles are used as catalysts in many chemical reactions (Thompson *et al.,* 2007). The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a reaction (nitrogen oxides). Gold nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry.

Anticancer Activity of GNPs

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, antiallergic, 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.,* 2016*;* Rajeshkumar *et al.,* 2021).

Targeted Drug Delivery

Targeted drug delivery, also known as smart drug delivery, is a method of treatment that involves the increase in medicament in one or few body parts in comparison to others. Therefore, it delivers the medication only to areas of interest within the body. This offers an improved efficacy of treatment and also reduces side effects (Manish *et al.,* 2010). It differs from the conventional drug delivery system in that, it gets release in a dosage form while the former functions by the absorption of drug across biological membrane (Rani *et al.,* 2014). There are four principles requirements for a successful targeted drug delivery system: retain, evade, target and release, i.e., there should be proper loading of the drug into an appropriate drug delivery vehicle, it must possess an ability to escape the body's secretions that may degrade it, leading to a long residence time in circulation and thereby reaching the site of interest and, should release the drug at the specific site within the time that calls for effective drug functioning. Different sites of interest within the body necessitate the use of different drug delivery systems, depending upon the route to be followed (Bae *et al.,* 2011).

Nanoparticles in Targeted Drug Delivery

NPs can usually be concentrated in the tumor area due to abnormal leaky vasculature of tumor tissue which is also known as enhanced permeation and retention (EPR) effect. EPR effect facilitates transition of nanovectors with size of 400 nm in diameter into the surrounding tumor tissue (Sinha *et al.,* 2006*;* Smith *et al.,* 2008). Therefore, passive targeting depends on some pathophysiological features of tumor tissue including the abnormal vasculature, temperature, pH, and surface charge of tumor cells (Prabhu *et al.,* 2015). It is evident that some physicochemical properties of nanocarriers such as size, surface charge, molecular weight, and hydrophobic or hydrophilic feature are crucial for passive targeting. Although passive targeting is interesting approach, however, it suffers from the serious limitations such as inefficient drug diffusion into tumor cells, the random nature of targeting, and the lack of EPR effect in some tumors. One of the best ways to solve the problems of passive targeting is conjugation of ligands of tumor specific biomarkers with nanocarriers which is

referred as active targeting. There are several targeting moieties such as monoclonal antibodies and their variable fragments, peptides, aptamers, vitamins, and carbohydrates (Danhier *et al.,* 2010). It is evident that tumor specific biomarkers should be overexpressed on tumor cells to reach high specificity.

Synthesis of GNPs

A variety of techniques, including chemical, physical, biological, thermal, electrochemical and sonochemical pathways, have so far been introduced for the synthesis of GNPs *(*Mandal *et al.,* 2014*;* Nakanishi *et al.,* 2005*;* Porta *et al.,* 2003*;* Yu *et al.,* 1997).

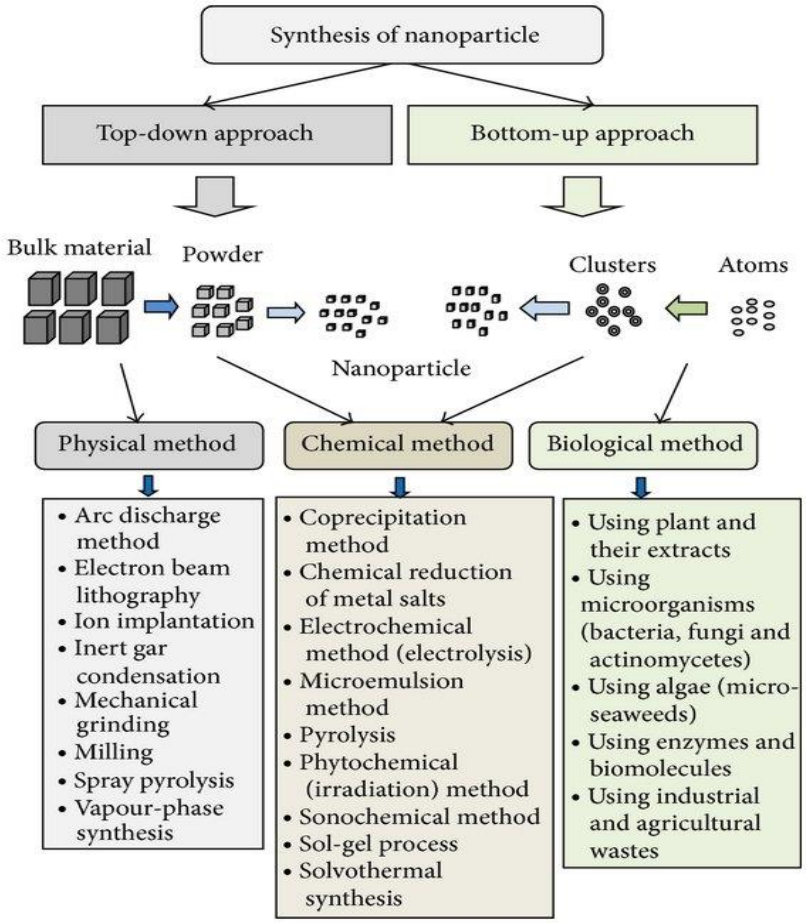


Figure 1. Synthesis of Nanoparticles

Chemical Method

Generally, the preparation of AuNPs by the chemical reduction method includes two main parts: (1) reduction by agents, for instance borohydrides, aminoboranes, 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).

Most common chemical methods are:

- Citrate Synthesis
- Turkevich method
- Wet chemical synthesis
- Chemical reduction method

Physical Method

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 colours (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 (Hammami *et al.,* 2021).

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 (Al-Saffar *et al.,* 2018).

Most common physical methods are:

- UV irradiation
- Laser ablation
- Plasma synthesis

Biological Method

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 (Noruzi *et al.,* 2011*;* Shankar *et al.,* 2004). 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 centre 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*;* Singh *et al.,* 2013).

Microbial Synthesis of GNPs

Synthesis by Bacteria

Among microorganisms, prokaryotes have received the most attention in the area of AuNP synthesis (Whiteley *et al.,* 2011). For the first time microbial synthesis of AuNPs was reported in Bacillus subtilis 168 which revealed the presence of 5–25 nm octahedral NPs inside the cell wall (Beveridge & Murray, 1980). In Rhodopseudomonas capsulata, spherical AuNPs with 10–20 nm range have been observed (He *et al.,* 2007) at lower concentration and nanowires with network at higher concentration (He *et al.,* 2008). Six cyanobacteria have been reported for production of AuNPs.

Plectonema sp. (He *et al.,* 2008*;* Schröfel *et al.,* 2011), Anabaena sp., Calothrix sp., and Leptolyngbya sp. have been exploited for the AuNP synthesis (Brayner *et al.,* 2007). Single-cell protein of Spirulina platensis was also shown to produce AuNPs and Au core–Ag shell NPs (Govindaraju *et al.,* 2008). If one tries to group the AuNP producing bacteria according to 9th edition of Bergey's Manual of Systematic Bacteriology (Garrity *et al.,* 2004), the members belonging to groups glidobacteria, and beta, epsilon and zeta proteobacteria have not been reported so far.

Synthesis by Fungi

Fungi appear to be more promising for large scale production of NPs as they are simpler to grow both in the laboratory and at an industrial scale as well as secrete large quantity of proteins. Besides this, fungi synthesize NPs of defined dimensions with good monodispersity (Ahmad *et al.,* 2002*;* Das & Marsili *et al.,* 2010; Mukherjee *et al.,* 2001). Different fungal species, e.g., Fusarium oxysporum, Verticillium sp. have been reported (Ahmad *et al.,* 2002*;* Kumar *et al.,* 2007*;* Mandal *et al.,* 2006) to synthesize NPs either intra or extracellularly. Shankar et al. (2004) (Shankar *et al.,* 2004) demonstrated that gold nanoplates can be synthesized by using fungal extracts. Yeasts like Pichia jadinii and Yarrowia lipolytica were also shown to have a good potential for synthesis of AuNPs (Agnihotri *et al.,* 2009*;* Gericke *et al.,* 2006) and they are now under the specialized exploration to engineer AuNPs. The yeast strains are having many advantages over bacteria for the bulk production of NPs as yeasts are easy to handle in laboratory conditions, synthesize high quantity of enzymes and grow rapidly employing simple nutrients (Singh *et al.,* 2014).

Plant Extract Mediated / Green Synthesis of GNPs

Nature has devised various processes for the synthesis of nano and micro length scaled inorganic materials which have contributed to the development of a relatively new and largely unexplored area of research based on the biosynthesis of the nanomaterials. Synthesis using bio-organisms is compatible with green chemistry principles. “Green synthesis” of nanoparticles makes use of environmentally friendly, non-toxic, and safe reagents. Nanoparticles synthesized using biological techniques or green technology have diverse natures, with greater stability and appropriate dimensions since they are synthesized using a one-step procedure (Parveen *et al.,* 2016).

Nature is rich with a wellspring of plants which has the advantage of a low cost, high reproducibility, eco-friendly and precise purification process compared to other environmentally friendly biological methods. The green pathways with the use of plant extracts as reducing agents and stabilizers for the preparation of gold nanoparticles has increased interest (Hammami *et al.,* 2021*;* Qiao *et al.,* 2021).

It's a direct method involves the selection of specific sections depending on the type of plants. For example, collect the root part of *Euphorbia fischeriana* plant which in general used as antioxidant, the pulp part of *Punicagranatum* as antimicrobial, the leaf from *A. noeanum* as antibacterial, and the juice of *Papaya* as Sensing L-Lys (Khatua *et al.,* 2020*;* Qiao *et al.,* 2021*;* Shahriari *et al.,* 2019). Polyphenols, flavonoids, sugar reduction, polysaccharides, alkaloids, amino acids, vitamins, ketones, phenols, and proteins are examples of plant extract biomolecules (Siddiqi & Husen*,* 2017). A plant containing at least one of the above-mentioned chemicals that reduces the metal ion to elemental metal must always be chosen for biosynthesis. First, reduce $Au^{3+}$to Au^{0}, then meditate and stabilized AuNPs by covering the outer surface of gold in order to prevent aggregation (Qiao *et al.,* 2021).

Withania somnifera Mediated Synthesis of GNPs

In present study anisotropic gold nanoparticles were synthesized using *Withania somnifera* leaf ethanolic extract and demonstrated their potential in absorption of infrared rays. These green synthesized nanoparticles were examined by ultraviolet visible spectroscopy, transmission electron microscopy (TEM), energy dispersive X-ray analysis (EDAX), and Fourier transform infrared (FTIR) spectroscopy to determine their size and shape. Withania somnifera contains various metabolites like withanolide D and withaferin D. Alkaloids, steroids, saponins, phenolics, flavonoids, phytophenols, and glycosides are also present in the plant which mediates the synthesis of golds nanoparticles. These metabolites act as reducing and capping agent which is necessary for the synthesis of GNPs. Withania somnifera mediated synthesis of GNPs are considered as eco-friendly and fast method. (Bindhani *et al.,* 2014).

Stabilization and Functionalization of GNPs

For the successful application of GNPs, they must be stabilized to maintain their individuality upon the changing of the dispersion medium. For this task, self-assembled monolayers (SAMs) of polymers are frequently used as sterically

stabilizing agent. SAMs are thin monolayers of organic molecules that have end group that spontaneously and selectively interact with metal or semiconductor substrates (Kumar *et al.,* 2007). GNPs can be modified with bifunctional ligands by place exchange reaction to introduce e.g., bromide, ferrcene, hydroxyl and carboxyl functional groups.

Commonly found carboxylic groups can be reacted with primary amines by means of a condensation reaction to yield amide bond. For this, a water soluble carbondimide (e.g., EDC) is commonly used. Often forming an intermediate compound with the carboxylic moiety, the activated groups are reactive towards primary amines. In the case of primary amines present on the particles surface, active easter compounds (N hydroxyl- succinimidyl; NHS) can be used to equally form amide bonds, one example is succinimidyl-4-(N-maleimidomethyl) cyclohexane-1- carboxylate (SMCC) containing an NH's group reacting with primary amines, converting them to malaeimides that are reactive towards thiols (Yang *et al.,* 2007). Functionalization refers to the surface modification of NPs, which includes conjugation of chemicals (Herranz *et al.,* 2012) or bio molecules on to the surface like folic acid, biotin molecules, oligo nucleotides, peptides, antibodies, etc., to enhance the properties and hit the target with high precision (Aravind *et al.,* 2012). In addition to this, the functionalized NPs have good physical properties, anti-corrosion, anti-agglomeration and non-invasive characteristics. Intense researches have been carried out to functionalize the NPs to enhance its overall efficiency and modality (Subbiah *et al.,* 2010). Functionalization of NPs allows us to inculcate properties that we are specifically interested to incorporate in NPs, thus, it can be done to aid a specific imaging modality approach. It not only helps in getting better image quality, but also increases the applicability and functionality of the imaging modality. In various researches, functionalization of NPs is also found to be done with another NP for enhancing its characteristics (Thiruppathi *et al.,* 2017).

Characterisation of Nanoparticles

Two of the main parameters studied in the characterization of NPs are size and shape. We can also measure size distribution, degree of aggregation, surface charge and surface area, and to some extent evaluate the surface chemistry. Size, size distribution and organic ligands present on the surface of the particles may affect other properties and possible applications of the NPs. In addition, the crystal structure of the NPs and

their chemical composition are thoroughly investigated as a first step after nanoparticle synthesis (Mourdikoudis *et al.,* 2018).

Scanning Electron Microscope

The Scanning Electron Microscope gives surface morphology of material. It generates a beam of electrons in a vacuum. That beam is collimated by electromagnetic condenser lenses, focused by an objective lens, and scanned across the surface of the sample by electromagnetic deflection coils. The primary imaging method is by collecting secondary electrons that are released by the sample. The secondary electrons are detected by a scintillation material that produces flashes of light from the electrons. The light flashes are then detected and amplified by a photomultiplier tube. By correlating the sample scan position with the resulting signal, an image can be formed that is strikingly similar to what would be seen through an optical microscope. The shadowing and illumination show a quite natural looking surface topography.

The electron gun in a Scanning Electron Microscope is the source for the electron beam used to probe the sample. Electrons are emitted from a cathode, accelerated by passage through electrical fields and focused to a first optical image of the source. The size and shape of the apparent source, beam acceleration, and current are the primary determining factors in the performance and resolution of a scanning electron microscope. A bent tungsten wire filament, with a diameter of around 100 micrometres, is spot welded to metal posts. These posts are embedded in a ceramic holder and extend out the other side to provide electrical connections. In operation, the filament will be heated by passing an electrical current through it. Optimum filament temperature for the thermionic emission of electrons is around 2700∞K. The accelerating voltage, generally between −500 Volts and −50,000 Volts DC, is applied to the Wehnelt cylinder (Srivastava, 2012).

Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) is used for particle size, shape, and morphology. TEM is an imaging technique whereby a beam of photographic film (see electron microscope), or to be detected by a CCD camera. Electrons are generated by a process known as thermionic discharge in the same manner as the cathode in a cathode-ray tube or by field emission; they are then accelerated by an electric field and focused by electrical and magnetic fields onto the sample. Another type of TEM

is the Scanning Transmission Electron Microscope (STEM), where the beam can be restored across the sample to form the image. Formation of Image in the TEM A crystalline material interacts with the electron beam mostly by absorption, although the intensity of the transmitted beam is still affected by the volume and density of the material through which it passes. The intensity of the diffraction depends on the orientation of the planes of atoms in a crystal structure (Werner *et al.,* 1997).

Fourier transform infrared (FTIR) spectroscopy

Infrared (IR) or Fourier transform infrared (FTIR) spectroscopy has a large application range, from the analysis of small molecules or molecular complexes to the analysis of cells or tissues. The imaging of tissues is one of the recent developments of infrared spectroscopy, taking advantage of infrared microscopy and of the use of synchrotron IR radiation. It is used for the mapping of cellular components (carbohydrates, lipids, proteins) to identify abnormal cells (Levin *et al.,* 2005).

Molecular absorption of electromagnetic radiation in the infrared region of the spectrum promotes transitions between the rotational and vibrational energy levels of the ground (lowest) electronic energy state. This is in contrast to absorption of the energetically more powerful visible and ultraviolet radiation, which induces transitions between vibrational and rotational energy levels of different electronic levels. Infrared spectroscopy is concerned primarily with molecular vibrations, as transitions between individual rotational states can be measured only in the infrared spectra of small molecules in the gas phase (Ismail *et al.,* 1997).

FTIR difference spectroscopy has been widely applied in photosynthesis research and related areas. This approach gives complementary information to the three-dimensional structural data obtained by X-ray diffraction (or Nuclear Magnetic Resonance, NMR). The analysis of active sites in proteins by means of reaction-induced FTIR difference spectroscopy gives information on minute structural changes, hydrogen-bonding interactions, and proton transfer reactions, which are often beyond the sensitivity of Xray diffraction analyses. Moreover, time-resolved techniques, with time resolution now up to the femtosecond range (di Donato *et al.,* 2008).

UV- VIS Absorption Spectroscopy

UV-Vis Absorption Spectroscopy gives UV absorption of the amorphous gels and crystalline ceramic samples heated at different temperatures. Many molecules absorb

visible or ultraviolet light. The absorbance of a solution is directly proportional to attenuation of the beam, i.e., it increases as attenuation of the beam increases. Absorbance is also directly proportional to the path length “b” and the concentration “c” of the absorbing species (Thomas *et al.,* 2008).

Beer Lambert’s Law states that A = εbc Where ε is a constant of proportionality, called the absorbtivity. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. When an atom or molecule absorbs energy, electrons are excited from their ground state to an excited state. In a molecule, the atoms can vibrate and rotate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level. The absorption of visible or UV radiation occurs to the excitation of outer electrons.

There are three types of electronic transition which are given as follows:

- Transitions involving d and f electrons
- Transitions involving p, s and n electrons
- Transitions involving charge-transfer electrons

Zeta Potential

In an ionic solution, nanoparticles with a net charge will have a layer of ions (of opposite charge) strongly bound to their surface; this is referred to as the Stern layer. A second diffuse outer layer is comprised of loosely associated ions. These two layers are collectively called the electrical double layer. As the particle moves (due to Brownian diffusion or applied force), a distinction is created between ions in the diffuse layer that move with the nanoparticle and ions that remain with the bulk dispersant. The electrostatic potential at this “slipping plane” boundary is called the zeta potential and is related to the surface charge of the nanoparticle. In zeta potential measurements, an electrical field is applied across the sample and the movement of the nanoparticles (electrophoretic mobility) is measured by laser doppler velocimetry (LDV) (Clogston *et al.,* 2011). Nanoparticles with a zeta potential between −10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than −30 mV are considered strongly cationic and strongly anionic, respectively. Since most cellular membranes are negatively charged,

zeta potential can affect a nanoparticle's tendency to permeate membranes, with cationic particles generally displaying more toxicity associated with cell wall disruption (Dukhovich *et al.,* 2004).

Dynamic Light Scattering

Dynamic light scattering (DLS) particle sizing characterizes the temporal structure of particles' Brownian motion in liquid suspension, which carries critical information about the size of the particles. By measuring the temporal structure instead of angular distribution, DLS is able to measure particles as small as a few nanometres, much smaller than the wavelength of light used (Yin, 2012). Dynamic light scattering (DLS) is based on the Brownian motion of dispersed particles. When particles are dispersed in a liquid they move randomly in all directions. The principle of Brownian motion is that particles are constantly colliding with solvent molecules. These collisions cause a certain amount of energy to be transferred, which induces particle movement. The energy transfer is more or less constant and therefore has a greater effect on smaller particles. As a result, smaller particles are moving at higher speeds than larger particles. If you know all other parameters which have an influence on particle movement, you can determine the hydrodynamic diameter by measuring the speed of the particles. The relation between the speed of the particles and the particle size is given by the Stokes-Einstein equation (Takahashi *et al.,* 2008).

OBJECTIVE

Objectives

- Biogenic synthesis of gold nanoparticles by using *Withania somnifera* leaf extract.
- Characterization of biogenically synthesized gold nanoparticles by UV-Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential.
- Evaluation of the potential 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

Withania somnifera plant was a kind gift 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.

Withania somnifera- 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 hrs 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 *Withania somnifera* 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 colour change. 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 (*Mishra et al., 2022*).

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 WS-extract WS-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 cytomorphological changes in HeLa cells

HeLa cells were pre-treated with different concentrations of each, WS- Extract and WS-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 WS-extract and WS-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 WS-extract and WS-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

Withania somnifera Mediated synthesis of AuNPs (WS-AuNPs)

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

Characterization of WS-AuNPs

The phyto-constituents in *Withania somnifera* 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 WS-AuNPs. The change in colour from light green to ruby red indicated the successful synthesis of WS-AuNPs, and the result of SPR band confirm that at 528 and however there was no discernible peek for *Withania somnifera* 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 WS-AuNPs had an average particle size of 56.44 d nm as shown in figure. Furthermore, the Zeta potential of the prepared WS-AuNPs was observed at the room temperature, to be a -19.1 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.

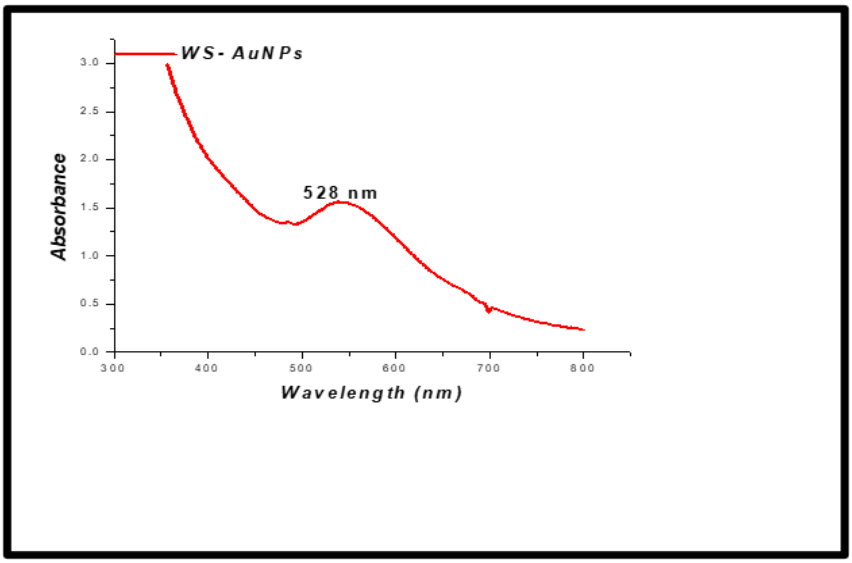


Figure 2: Characterisation of WS-AuNPs under UV-Visible spectra (528 nm).

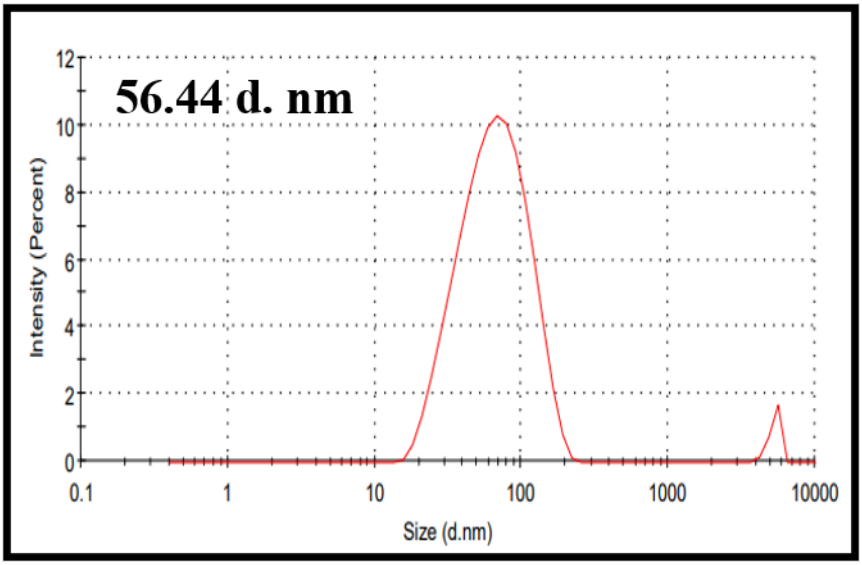


Figure 3: DLS profile of WS-AuNPs showing size of 56.44 d.nm.

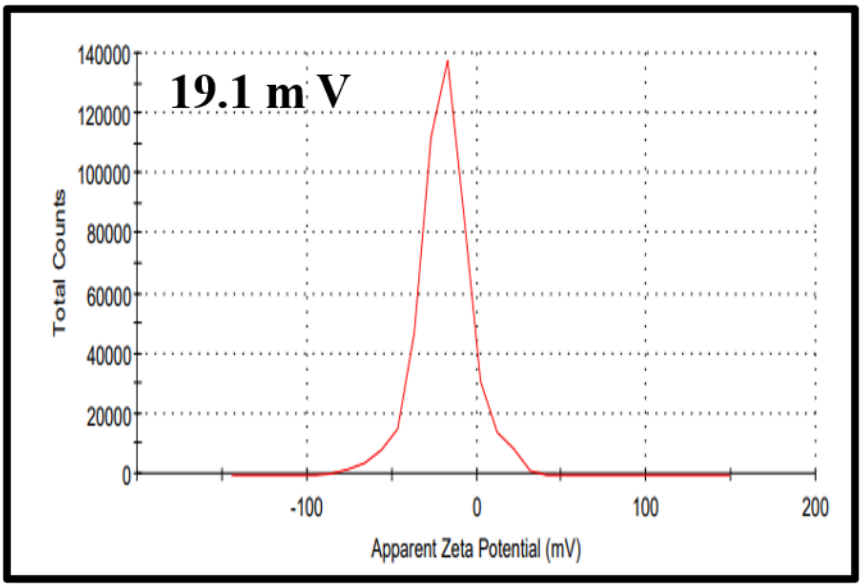


Figure 4: Zeta potential of WS-AuNPs confirmed the stability at -19.1 mV.

Determination of cytomorphological changes in HeLa cells

Morphological analysis of the WS-extract and WS-AuNPs, 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 WS-extract (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml) and WS-AuNPs, (20µg/ml, 40µg/ml, 80µg/ml) concentrations for 24 h. In the presence of different doses WS- extract and WS-AuNPs, 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 cells. 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.

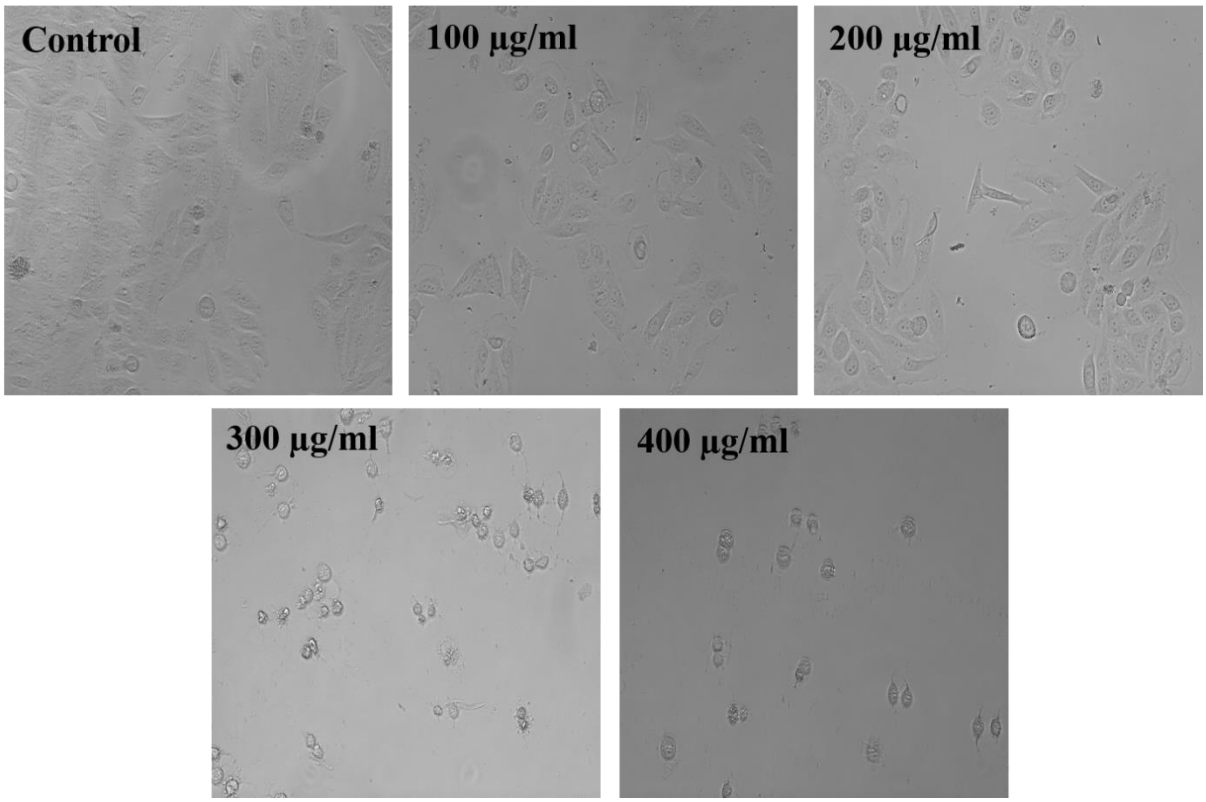


Figure 5: The phase contrast microscopy of HeLa cell treated with either vehicle control or different doses of *Withania somnifera* WS-extract (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml) for 24 h in a time and dose dependent manner.

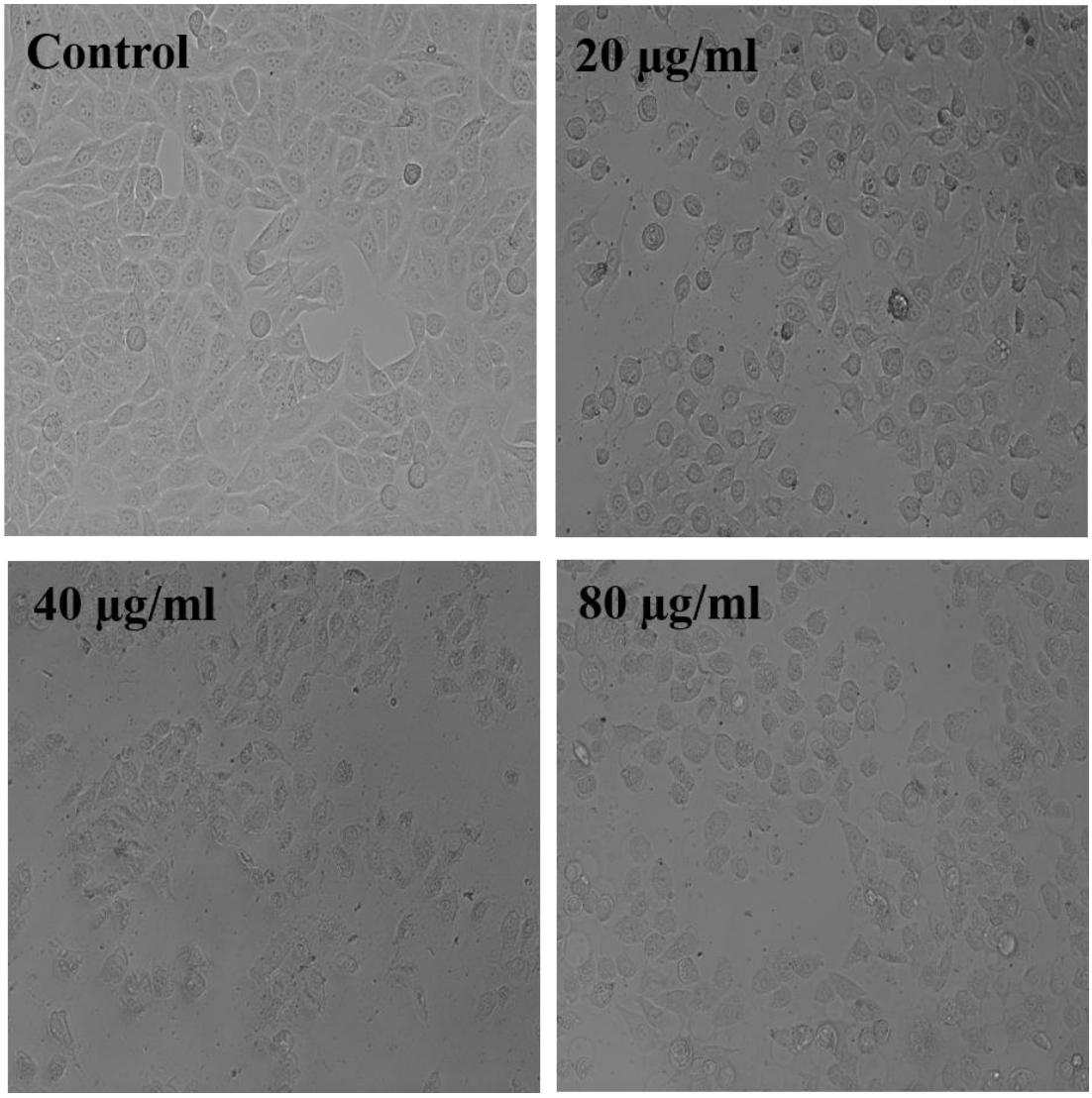
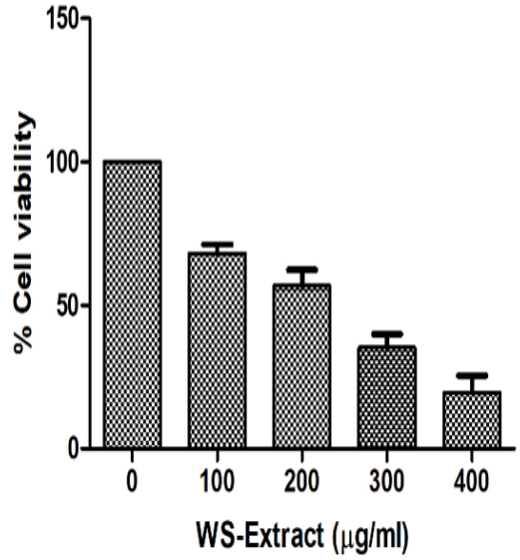
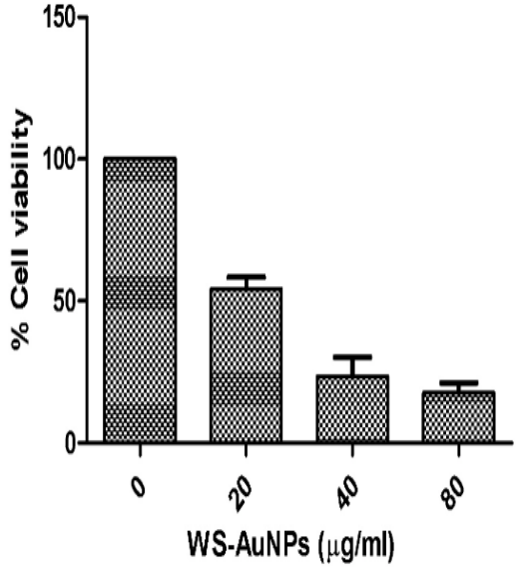


Figure 6: The phase contrast microscopy of HeLa cell treated with either vehicle control or different doses/concentration of WS-AuNPs (20µg/ml, 40µg/ml, 80µg/ml) for 24 h in a time and dose dependent manner.

In vitro cytotoxicity of WS-extract and WS-AuNPs

To evaluate the sensitivity of cervical cancer cells to these drugs, HeLa cells were treated with different doses of WS- extract and WS-AuNPs, for 24 h followed by MTT assay. Our results showed that, after 24 h of treatment, AuNPs at a concentration IC50=142.40±1.13µg/ml reduced growth of HeLa cells by 50%, while inhibition of 50% viability of HeLa cells was observed at IC50=26±1.19µg/ml WS-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.





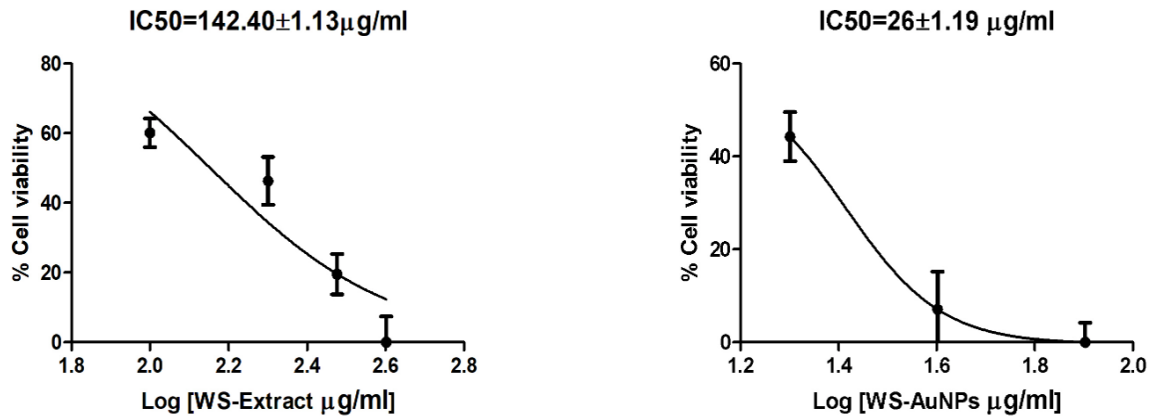


Figure 7: Percent cell viability of HeLa cells treated with different doses of *Withania somnifera* (100-400µg/ml) assessed by MTT Assay 24h. Graph showed that of *Withania somnifera* extract exhibited IC50 value 142.40µg/ml at 24 h against HeLa cervical cancer cell. Percent cell viability of HeLa cells treated with different doses WS-AuNPs (20,40,80µg/ml) assessed by MTT Assay 24h. The result represented are the mean ±SEM of three independent experiment performed in triplicate. Graph showed that WS-AuNPs exhibited IC50 value 26±µg/ml at 24 h, against HeLa cervical cancer cell. The result represented are the mean ±SEM of three independent experiment performed in triplicate.

CONCLUSION

Conclusion and future Perspectives

In this study we showed a Leaf extract mediated green synthesis of gold nanoparticles from *Withania somnifera* plant and their characterization, anticancer property analysis. This study investigates an efficient and sustainable route of AuNP preparation from 1mM aqueous HAuCl4 using leaf extracts of *Withania somnifera* 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 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.

REFERENCE

References

Agnihotri, M., Joshi, S., Kumar, A. R., Zinjarde, S., & Kulkarni, S. (2009). Biosynthesis of gold nanoparticles by the tropical marine yeast Yarrowia lipolytica NCIM 3589. *Materials Letters*, *63*(15), 1231–1234.

Ahmad, A., Mukherjee, P., Mandal, D., Senapati, S., Khan, M. I., Kumar, R., & Sastry, M. (2002). Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, Fusarium oxysporum. *Journal of the American Chemical Society*, *124*(41), 12108–12109.

Alanazi, F. K., Radwan, A. A., & Alsarra, I. A. (2010). Biopharmaceutical applications of nanogold. *Saudi Pharmaceutical Journal*, *18*(4), 179–193.

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., & Islam, K. N. (2012). Gold nanoparticle sensor for the visual detection of pork adulteration in meatball formulation. *Journal of Nanomaterials*, *2012*.

Aljarba, N. H., Imtiaz, S., Anwar, N., Alanazi, I. S., & Alkahtani, S. (2022). Anticancer and microbial activities of gold nanoparticles: A mechanistic review. *Journal of King Saud University - Science*, *34*(4), 101907. https://doi.org/10.1016/j.jksus.2022.101907

Allhoff, F. (2007). On the Autonomy and Justification of Nanoethics. *NanoEthics*, *1*(3), 185–210. https://doi.org/10.1007/s11569-007-0018-3

Al-Saffar, A., Yousif, E., & Alshanon, A. (2018). *Gold Nanoparticles Detection by Plants Extracts – A Review*. *12*, 41–58.

An official website of the United States government. (n.d.). *National Nanotechnology Initiative (NNI)*. Retrieved June 19, 2022, from https://www.nano.gov/

Aravind, A., Jeyamohan, P., Nair, R., Veeranarayanan, S., Nagaoka, Y., Yoshida, Y., Maekawa, T., & Kumar, D. S. (2012). AS1411 aptamer tagged PLGA-lecithin-PEG nanoparticles for tumor cell targeting and drug delivery. *Biotechnology and Bioengineering*, *109*(11), 2920–2931.

Bae, Y. H., & Park, K. (2011). Targeted drug delivery to tumors: myths, reality and possibility. *Journal of Controlled Release*, *153*(3), 198.

Banerjee, K., Das, S., Choudhury, P., Ghosh, S., Baral, R., & Choudhuri, S. K. (2017). A novel approach of synthesizing and evaluating the anticancer potential of silver oxide nanoparticles in vitro. *Chemotherapy*, *62*(5), 279–289.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2019). The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. *Molecules*, *25*(1), 112. https://doi.org/10.3390/molecules25010112

Beveridge, T. J., & Murray, R. G. (1980). Sites of metal deposition in the cell wall of Bacillus subtilis. *Journal of Bacteriology*, *141*(2), 876–887.

Bhatia, P., Rattan, S. I. S., Cavallius, J., & Clark, B. F. C. (1987). Withania somnifera(Ashwagandha), a so-called rejuvenator, inhibits growth and macromolecular synthesis of human cells. *Medical Science Research*, *15*(9), 515–516.

Bhattacharya, R., & Mukherjee, P. (2008). Biological properties of “naked” metal nanoparticles. *Advanced Drug Delivery Reviews*, *60*(11), 1289–1306.

Bindhani, B. K. (2014). Green synthesis and Characterization of Gold Nanoparticles Using Leaf Extracts of Withania somnifera (Linn.) (Ashwagandha). *International Journal of Materials Science and Applications*, *3*, 279. https://doi.org/10.11648/j.ijmsa.20140306.11

Bindhani, B. K., & Panigrahi, A. K. (2014). Green synthesis and characterization of gold nanoparticles using leaf extracts of Withania somnifera (Linn.)(Ashwagandha). *Int. J. Mater. Sci. Appl*, *3*(6), 279–284.

Bogart, L. K., Pourroy, G., Murphy, C. J., Puntes, V., Pellegrino, T., Rosenblum, D., Peer, D., & Lévy, R. (2014). Nanoparticles for imaging, sensing, and therapeutic intervention. *ACS Nano*, *8*(4), 3107–3122.

Braga, D., & Grepioni, F. (1994). From molecule to molecular aggregation: clusters and crystals of clusters. *Accounts of Chemical Research*, *27*(2), 51–56. https://doi.org/10.1021/ar00038a003

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality

worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424.

Brayner, R., Barberousse, H., Hemadi, M., Djedjat, C., Yéprémian, C., Coradin, T., Livage, J., Fiévet, F., & Couté, A. (2007). Cyanobacteria as Bioreactors for the Synthesis of Au, Ag, Pd, and Pt Nanoparticles via an Enzyme-Mediated Route. *Journal of Nanoscience and Nanotechnology*, *7*(8), 2696–2708. https://doi.org/10.1166/jnn.2007.600

Brown, S. D., Nativo, P., Smith, J.-A., Stirling, D., Edwards, P. R., Venugopal, B., Flint, D. J., Plumb, J. A., Graham, D., & Wheate, N. J. (2010). Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *Journal of the American Chemical Society*, *132*(13), 4678–4684.

Calzolai, L., Franchini, F., Gilliland, D., & Rossi, F. (2010). Protein--nanoparticle interaction: identification of the ubiquitin--gold nanoparticle interaction site. *Nano Letters*, *10*(8), 3101–3105. https://doi.org/10.1021/nl101746v

Chen, G., Roy, I., Yang, C., & Prasad, P. N. (2016). Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy. *Chemical Reviews*, *116*(5), 2826–2885.

Chritof. M. (2004). *Nanobiotechnology*. Wiley. https://doi.org/10.1002/3527602453

Clogston, J. D., & Patri, A. K. (2011). Zeta potential measurement. In *Characterization of nanoparticles intended for drug delivery* (pp. 63–70). Springer.

Danhier, F., Feron, O., & Préat, V. (2010). To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of Controlled Release*, *148*(2), 135–146.

Das, S. K., & Marsili, E. (2010). A green chemical approach for the synthesis of gold nanoparticles: characterization and mechanistic aspect. *Reviews in Environmental Science and Bio/Technology*, *9*(3), 199–204.

Dean, S., Mansoori, G., & Fauzi Soelaiman, T. (2005). Nanotechnology — An Introduction for the Standards Community. *Journal of ASTM International*, *2*(6), 13110. https://doi.org/10.1520/JAI13110

Deb, S., Patra, H. K., Lahiri, P., Dasgupta, A. K., Chakrabarti, K., & Chaudhuri, U. (2011). Multistability in platelets and their response to gold nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(4), 376–384.

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. *Arabian Journal of Chemistry*, *10*, S1193–S1199. https://doi.org/10.1016/j.arabjc.2013.02.015

di Donato, M., Cohen, R. O., Diner, B. A., Breton, J., van Grondelle, R., & Groot, M. L. (2008). Primary charge separation in the photosystem II core from Synechocystis: a comparison of femtosecond visible/midinfrared pump-probe spectra of wild-type and two P680 mutants. *Biophysical Journal*, *94*(12), 4783–4795.

di Guglielmo, C., López, D. R., de Lapuente, J., Mallafre, J. M. L., & Suàrez, M. B. (2010). Embryotoxicity of cobalt ferrite and gold nanoparticles: a first in vitro approach. *Reproductive Toxicology*, *30*(2), 271–276.

Dubchak, S., Ogar, A., Mietelski, J. W., & Turnau, K. (2010). Influence of silver and titanium nanoparticles on arbuscular mycorrhiza colonization and accumulation of radiocaesium in Helianthus annuus. *Spanish Journal of Agricultural Research*, *1*, 103–108.

Dukhovich, F. S. (2004). *Molecular recognition: Pharmacological aspects*. Nova Publishers.

Eck, W., Craig, G., Sigdel, A., Ritter, G., Old, L. J., Tang, L., Brennan, M. F., Allen, P. J., & Mason, M. D. (2008). PEGylated gold nanoparticles conjugated to monoclonal F19 antibodies as targeted labeling agents for human pancreatic carcinoma tissue. *ACS Nano*, *2*(11), 2263–2272. https://doi.org/10.1021/nn800429d

Eleraky, N. E., Allam, A., Hassan, S. B., & Omar, M. M. (2020). Nanomedicine Fight against Antibacterial Resistance: An Overview of the Recent Pharmaceutical Innovations. *Pharmaceutics*, *12*(2). https://doi.org/10.3390/pharmaceutics12020142

El-Sayed, I. H., Huang, X., & El-Sayed, M. A. (2005). Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. *Nano Letters*, *5*(5), 829–834. https://doi.org/10.1021/nl050074e

Eustis, S., & el-Sayed, M. A. (2006). Why gold nanoparticles are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chemical Society Reviews*, *35*(3), 209–217. https://doi.org/10.1039/b514191e

Faraday. M. (1857). X. The Bakerian Lecture. —Experimental relations of gold (and other metals) to light. *Philosophical Transactions of the Royal Society of London*, *147*, 145–181. https://doi.org/10.1098/rstl.1857.0011

Franco, E. L. (1991). The sexually transmitted disease model for cervical cancer: incoherent epidemiologic findings and the role of misclassification of human papillomavirus infection. *Epidemiology*, 98–106.

Ganeshkumar, M., Sastry, T. P., Kumar, M. S., Dinesh, M. G., Kannappan, S., & Suguna, L. (2012). Sun light mediated synthesis of gold nanoparticles as carrier for 6-mercaptopurine: Preparation, characterization and toxicity studies in zebrafish embryo model. *Materials Research Bulletin*, *47*(9), 2113–2119.

Gao, Z., Zhang, L., & Sun, Y. (2012). Nanotechnology applied to overcome tumor drug resistance. *Journal of Controlled Release*, *162*(1), 45–55.

Garrity, G. M., Bell, J. A., & Lilburn, T. G. (2004). Taxonomic outline of the prokaryotes. Bergey's manual of systematic bacteriology. *New York*.

Geetha, R., Ashokkumar, T., Tamilselvan, S., Govindaraju, K., Sadiq, M., & Singaravelu, G. (2013). Green synthesis of gold nanoparticles and their anticancer activity. *Cancer Nanotechnology*, *4*(4), 91–98.

Geiger, T. R., & Peeper, D. S. (2009). Metastasis mechanisms. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1796*(2), 293–308.

Gericke, M., & Pinches, A. (2006). Microbial production of gold nanoparticles. *Gold Bulletin*, *39*(1), 22–28.

Gnach, A., Lipinski, T., Bednarkiewicz, A., Rybka, J., & Capobianco, J. A. (2015). Upconverting nanoparticles: assessing the toxicity. *Chemical Society Reviews*, *44*(6), 1561–1584. https://doi.org/10.1039/C4CS00177J

Gomez-Romero, P. (2001). Hybrid organic–inorganic materials—in search of synergic activity. *Advanced Materials*, *13*(3), 163–174.

Govindaraju, K., Basha, S. K., Kumar, V. G., & Singaravelu, G. (2008). Silver, gold and bimetallic nanoparticles production using single-cell protein (Spirulina platensis) Geitler. *Journal of Materials Science*, *43*(15), 5115–5122. https://doi.org/10.1007/s10853-008-2745-4

Gunasinghe, N. P. A., Wells, A., Thompson, E. W., & Hugo, H. J. (2012). Mesenchymal–epithelial transition (MET) as a mechanism for metastatic colonisation in breast cancer. *Cancer and Metastasis Reviews*, *31*(3), 469–478.

Guo, S., Huang, Y., Jiang, Q., Sun, Y., Deng, L., Liang, Z., Du, Q., Xing, J., Zhao, Y., Wang, P. C., Dong, A., & Liang, X.-J. (2010). Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. *ACS Nano*, *4*(9), 5505–5511. https://doi.org/10.1021/nn101638u

Gupta, Y. K., Sharma, S. S., Rai, K., & Katiyar, C. K. (2001). Reversal of Paclitaxel Induced Neutropenia by Withania Sominifera in Mice. *Indian Journal of Physiology and Pharmacology*, *45*(2), 253–257.

Hammami, I., Alabdallah, N. M., jomaa, A. al, & kamoun, M. (2021a). Gold nanoparticles: Synthesis properties and applications. *Journal of King Saud University - Science*, *33*(7), 101560. https://doi.org/10.1016/j.jksus.2021.101560

Hammami, I., Alabdallah, N. M., jomaa, A. al, & kamoun, M. (2021b). Gold nanoparticles: Synthesis properties and applications. *Journal of King Saud University - Science*, *33*(7), 101560. https://doi.org/10.1016/j.jksus.2021.101560

He, S., Guo, Z., Zhang, Y., Zhang, S., Wang, J., & Gu, N. (2007). Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. *Materials Letters*, *61*(18), 3984–3987.

He, S., Zhang, Y., Guo, Z., & Gu, N. (2008). Biological synthesis of gold nanowires using extract of Rhodopseudomonas capsulata. *Biotechnology Progress*, *24*(2), 476–480.

Herranz, F., Pellico, J., & Ruiz-Cabello, J. (2012). Covalent functionalization of magnetic nanoparticles for biomedical imaging. *SPIE Newsroom*. https://doi.org/10.1117/2.1201209.004473

Huang, D., Liao, F., Molesa, S., Redinger, D., & Subramanian, V. (2003). Plastic-Compatible Low Resistance Printable Gold Nanoparticle Conductors for Flexible Electronics. *Journal of The Electrochemical Society*, *150*(7), G412. https://doi.org/10.1149/1.1582466

Hussain, S., & Amjad, M. (2021). A review on gold nanoparticles (GNPs) and their advancement in cancer therapy. *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, *7*(1), 19–25.

Iqbal, P., Preece, J. A., & Mendes, P. M. (2012). Nanotechnology: The “Top-Down” and “Bottom-Up” Approaches. In *Supramolecular Chemistry*. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470661345.smc195

Ismail, A. A., van de Voort, F. R., & Sedman, J. (1997). *Chapter 4 Fourier transform infrared spectroscopy: Principles and applications* (pp. 93–139). https://doi.org/10.1016/S0167-9244(97)80013-3

Jain, P. K., Lee, K. S., El-Sayed, I. H., & El-Sayed, M. A. (2006). Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *The Journal of Physical Chemistry. B*, *110*(14), 7238–7248. https://doi.org/10.1021/jp057170o

Kang, Y.-J., O’Connell, D. L., Tan, J., Lew, J.-B., Demers, A., Lotocki, R., Kliewer, E. v, Hacker, N. F., Jackson, M., & Delaney, G. P. (2015). Optimal uptake rates for initial treatments for cervical cancer in concordance with guidelines in Australia and Canada: Results from two large cancer facilities. *Cancer Epidemiology*, *39*(4), 600–611.

Ke, Y., al Aboody, M. S., Alturaiki, W., Alsagaby, S. A., Alfaiz, F. A., Veeraraghavan, V. P., & Mickymaray, S. (2019). Photosynthesized gold nanoparticles from

Catharanthus roseus induces caspase-mediated apoptosis in cervical cancer cells (HeLa). *Artificial Cells, Nanomedicine, and Biotechnology*, *47*(1), 1938–1946.

Khan, A. K., Rashid, R., Murtaza, G., & Zahra, A. (2014). Gold nanoparticles: synthesis and applications in drug delivery. *Tropical Journal of Pharmaceutical Research*, *13*(7), 1169–1177.

Khatua, A., Priyadarshini, E., Rajamani, P., Patel, A., Kumar, J., Naik, A., Saravanan, M., Barabadi, H., Prasad, A., Ghosh, Ilora, Paul, B., & Meena, R. (2020). Phytosynthesis, Characterization and Fungicidal Potential of Emerging Gold Nanoparticles Using Pongamia pinnata Leave Extract: A Novel Approach in Nanoparticle Synthesis. *Journal of Cluster Science*, *31*(1), 125–131. https://doi.org/10.1007/s10876-019-01624-6

Khlebtsov, N., & Dykman, L. (2011). Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. *Chemical Society Reviews*, *40*(3), 1647–1671. https://doi.org/10.1039/c0cs00018c

Klabunde, K. (2012). *Chemistry of free atoms and particles*. Elsevier.

Kulkarni, S. K., & Dhir, A. (2008). Withania somnifera: an Indian ginseng. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(5), 1093–1105.

Kumar, G. V. P., Shruthi, S., Vibha, B., Reddy, B. A. A., Kundu, T. K., & Narayana, C. (2007). Hot spots in Ag core− Au shell nanoparticles potent for surface-enhanced Raman scattering studies of biomolecules. *The Journal of Physical Chemistry C*, *111*(11), 4388–4392.

Kumar, H., Venkatesh, N., Bhowmik, H., & Kuila, A. (2018). Metallic nanoparticle: a review. *Biomedical Journal of Scientific & Technical Research*, *4*(2), 3765–3775.

Kumar, S., Gandhi, K. S., & Kumar, R. (2007). Modeling of formation of gold nanoparticles by citrate method. *Industrial & Engineering Chemistry Research*, *46*(10), 3128–3136.

Levin, I. W., & Bhargava, R. (2005). Fourier transform infrared vibrational spectroscopic imaging: integrating microscopy and molecular recognition. *Annual Review of Physical Chemistry*, *56*, 429.

Lorusso, D., Petrelli, F., Coinu, A., Raspagliesi, F., & Barni, S. (2014). A systematic review comparing cisplatin and carboplatin plus paclitaxel-based chemotherapy for recurrent or metastatic cervical cancer. *Gynecologic Oncology*, *133*(1), 117–123.

Mandal, D., Bolander, M. E., Mukhopadhyay, D., Sarkar, G., & Mukherjee, P. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. *Applied Microbiology and Biotechnology*, *69*(5), 485–492.

Mandal, S. (2014). Synthesis of radioactive gold nanoparticle in surfactant medium. *Journal of Radioanalytical and Nuclear Chemistry*, *299*(3), 1209–1212.

Manish, G., & Vimukta, S. (2010). Targeted drug delivery system: A Review. *Res. J. Chem. Sci*, *1*.

Mishra, P., Ahmad, A., Al-Keridis, L. A., Alshammari, N., Alabdallah, N. M., Muzammil, K., Saeed, M., & Ansari, I. A. (2022). Doxorubicin-Conjugated Zinc Oxide Nanoparticles, Biogenically Synthesised Using a Fungus Aspergillus niger, Exhibit High Therapeutic Efficacy against Lung Cancer Cells. *Molecules*, *27*(8), 2590. https://doi.org/10.3390/molecules27082590

Mohanpuria, P., Rana, N. K., & Yadav, S. K. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. *Journal of Nanoparticle Research*, *10*(3), 507–517.

Moura, D., Souza, M. T., Liverani, L., Rella, G., Luz, G. M., Mano, J. F., & Boccaccini, A. R. (2017). Development of a bioactive glass-polymer composite for wound healing applications. *Materials Science and Engineering: C*, *76*, 224–232.

Mourdikoudis, S., Pallares, R. M., & Thanh, N. T. K. (2018). Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale*, *10*(27), 12871–12934. https://doi.org/10.1039/C8NR02278J

Moyano, D. F., & Rotello, V. M. (2011). Nano Meets Biology: Structure and Function at the Nanoparticle Interface. *Langmuir*, *27*(17), 10376–10385. https://doi.org/10.1021/la2004535

Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I., Ramani, R., Parischa, R., Ajayakumar, P. v., Alam, M., Sastry, M., & Kumar, R. (2001). Bioreduction of AuCl(4)(-) Ions by the Fungus, Verticillium sp. and Surface Trapping of the Gold Nanoparticles Formed D.M. and S.S. thank the Council of Scientific and Industrial Research (CSIR), Government of India, for financial assistance. *Angewandte Chemie (International Ed. in English)*, *40*(19), 3585–3588. https://doi.org/10.1002/1521-3773(20011001)40:19<3585::aid-anie3585>3.0.co;2-k

Nakanishi, M., Takatani, H., Kobayashi, Y., Hori, F., Taniguchi, R., Iwase, A., & Oshima, R. (2005). Characterization of binary gold/platinum nanoparticles prepared by sonochemistry technique. *Applied Surface Science*, *241*(1–2), 209–212.

Narayanan, R., & El-Sayed, M. A. (2004). Shape-dependent catalytic activity of platinum nanoparticles in colloidal solution. *Nano Letters*, *4*(7), 1343–1348.

Noruzi, M., Zare, D., Khoshnevisan, K., & Davoodi, D. (2011). Rapid green synthesis of gold nanoparticles using Rosa hybrida petal extract at room temperature. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *79*(5), 1461–1465.

Pal, R., Panigrahi, S., Bhattacharyya, D., & Chakraborti, A. S. (2013). Characterization of citrate capped gold nanoparticle-quercetin complex: Experimental and quantum chemical approach. *Journal of Molecular Structure*, *1046*, 153–163.

Park, S., & Hamad-Schifferli, K. (2010). Enhancement of in vitro translation by gold nanoparticle--DNA conjugates. *ACS Nano*, *4*(5), 2555–2560. https://doi.org/10.1021/nn100362m

Parveen, K., Banse, V., & Ledwani, L. (2016). Green synthesis of nanoparticles: their advantages and disadvantages. *AIP Conference Proceedings*, *1724*(1), 020048.

Patra, J. K., & Baek, K.-H. (2014). Green nanobiotechnology: factors affecting synthesis and characterization techniques. *Journal of Nanomaterials*, *2014*.

Peng, G., Tisch, U., Adams, O., Hakim, M., Shehada, N., Broza, Y. Y., Billan, S., Abdah-Bortnyak, R., Kuten, A., & Haick, H. (2009). Diagnosing lung cancer in

exhaled breath using gold nanoparticles. *Nature Nanotechnology*, *4*(10), 669–673. https://doi.org/10.1038/nnano.2009.235

Perrault, S. D., & Chan, W. C. W. (2010). In vivo assembly of nanoparticle components to improve targeted cancer imaging. *Proceedings of the National Academy of Sciences*, *107*(25), 11194–11199.

Porta, F., & Rossi, M. (2003). Gold nanostructured materials for the selective liquid phase catalytic oxidation. *Journal of Molecular Catalysis A: Chemical*, *204*, 553–559.

Prabhu, R. H., Patravale, V. B., & Joshi, M. D. (2015). Polymeric nanoparticles for targeted treatment in oncology: current insights. *International Journal of Nanomedicine*, *10*, 1001.

Prasad, S. R., Elango, K., Damayanthi, D., & Saranya, J. S. (2013). Formulation and evaluation of azathioprine loaded silver nanopartilces for the treatment of rheumatoid arthritis. *AJBPS*, *3*(23), 28–32.

Qian, L., Su, W., Wang, Y., Dang, M., Zhang, W., & Wang, C. (2019). Synthesis and characterization of gold nanoparticles from aqueous leaf extract of Alternanthera sessilis and its anticancer activity on cervical cancer cells (HeLa). *Artificial Cells, Nanomedicine, and Biotechnology*, *47*(1), 1173–1180.

Qiao, J., & Qi, L. (2021a). Recent progress in plant-gold nanoparticles fabrication methods and bio-applications. *Talanta*, *223*, 121396. https://doi.org/10.1016/j.talanta.2020.121396

Qiao, J., & Qi, L. (2021b). Recent progress in plant-gold nanoparticles fabrication methods and bio-applications. *Talanta*, *223*, 121396. https://doi.org/10.1016/j.talanta.2020.121396

Qiao, J., & Qi, L. (2021c). Recent progress in plant-gold nanoparticles fabrication methods and bio-applications. *Talanta*, *223*, 121396. https://doi.org/10.1016/j.talanta.2020.121396

Radu Repanovici Angela, M. (2004). *O istorie a tiparului si a tipariturilor*. Ed. Univ. Transilvania.

Rai, M., Jogee, P. S., Agarkar, G., & Santos, C. A. dos. (2016). Anticancer activities of Withania somnifera: Current research, formulations, and future perspectives. In *Pharmaceutical Biology* (Vol. 54, Issue 2, pp. 189–197). Taylor and Francis Ltd. https://doi.org/10.3109/13880209.2015.1027778

Rajeshkumar, S. (2016). Anticancer activity of eco-friendly gold nanoparticles against lung and liver cancer cells. *Journal of Genetic Engineering and Biotechnology*, *14*(1), 195–202. https://doi.org/10.1016/j.jgeb.2016.05.007

Rajeshkumar, S., Malarkodi, C., al Farraj, D. A., Soliman Elshikh, M., & Mohana Roopan, S. (2021). Employing sulphated polysaccharide (fucoidan) as medium for gold nanoparticles preparation and its anticancer study against HepG2 cell lines. *Materials Today Communications*, *26*, 101975. https://doi.org/10.1016/j.mtcomm.2020.101975

Rani, K., & Paliwal, S. (2014). A review on targeted drug delivery: Its entire focus on advanced therapeutics and diagnostics. *Sch. J. App. Med. Sci*, *2*(1C), 328–331.

Roy, R. v, Suman, S., Das, T. P., Luevano, J. E., & Damodaran, C. (2013). Withaferin A, a steroidal lactone from Withania somnifera, induces mitotic catastrophe and growth arrest in prostate cancer cells. *Journal of Natural Products*, *76*(10), 1909–1915.

Sau, T. K., Rogach, A. L., Jäckel, F., Klar, T. A., & Feldmann, J. (2010). Properties and applications of colloidal nonspherical noble metal nanoparticles. *Advanced Materials (Deerfield Beach, Fla.)*, *22*(16), 1805–1825. https://doi.org/10.1002/adma.200902557

Schröfel, A., Kratošová, G., Bohunická, M., Dobročka, E., & Vávra, I. (2011). Biosynthesis of gold nanoparticles using diatoms—silica-gold and EPS-gold bionanocomposite formation. *Journal of Nanoparticle Research*, *13*(8), 3207–3216. https://doi.org/10.1007/s11051-011-0221-6

Shahriari, M., Hemmati, S., Zangeneh, A., & Zangeneh, M. M. (2019). Biosynthesis of gold nanoparticles using *Allium noeanum* Reut. ex Regel leaves aqueous extract; characterization and analysis of their cytotoxicity, antioxidant, and antibacterial properties. *Applied Organometallic Chemistry*, *33*(11). https://doi.org/10.1002/aoc.5189

Shaikh, S. F., Mane, R. S., Min, B. K., Hwang, Y. J., & Joo, O. (2016). D-sorbitol-induced phase control of TiO2 nanoparticles and its application for dye-sensitized solar cells. *Scientific Reports*, *6*(1), 1–10.

Shankar, S. S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A., & Sastry, M. (2004a). Biological synthesis of triangular gold nanoprisms. *Nature Materials*, *3*(7), 482–488.

Shankar, S. S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A., & Sastry, M. (2004b). Biological synthesis of triangular gold nanoprisms. *Nature Materials*, *3*(7), 482–488.

Siddiqi, K. S., & Husen, A. (2017). Recent advances in plant-mediated engineered gold nanoparticles and their application in biological system. *Journal of Trace Elements in Medicine and Biology*, *40*, 10–23. https://doi.org/10.1016/j.jtemb.2016.11.012

Singh, M., Kalaivani, R., Manikandan, S., Sangeetha, N., & Kumaraguru, A. K. (2013). Facile green synthesis of variable metallic gold nanoparticle using Padina gymnospora, a brown marine macroalga. *Applied Nanoscience*, *3*(2), 145–151.

Singh, R., Smitha, M. S., & Singh, S. P. (2014). The Role of Nanotechnology in Combating Multi-Drug Resistant Bacteria. *Journal of Nanoscience and Nanotechnology*, *14*(7), 4745–4756. https://doi.org/10.1166/jnn.2014.9527

Sinha, R., Kim, G. J., Nie, S., & Shin, D. M. (2006). Nanotechnology in cancer therapeutics: bioconjugated nanoparticles for drug delivery. *Molecular Cancer Therapeutics*, *5*(8), 1909–1917.

Smith, A. M., Duan, H., Mohs, A. M., & Nie, S. (2008). Bioconjugated quantum dots for in vivo molecular and cellular imaging. *Advanced Drug Delivery Reviews*, *60*(11), 1226–1240.

Srivastava, R. (2012). Synthesis and Characterization Techniques of Nanomaterials. *International Journal of Green Nanotechnology*, *4*(1), 17–27. https://doi.org/10.1080/19430892.2012.654738

Strambeanu, N., Demetrovici, L., Dragos, D., & Lungu, M. (2015). Nanoparticles: Definition, classification and general physical properties. In *Nanoparticles' promises and risks* (pp. 3–8). Springer.

Stuchinskaya, T., Moreno, M., Cook, M. J., Edwards, D. R., & Russell, D. A. (2011). Targeted photodynamic therapy of breast cancer cells using antibody-phthalocyanine-gold nanoparticle conjugates. *Photochemical & Photobiological Sciences*, *10*(5), 822–831.

Subbiah, R., Veerapandian, M., & S Yun, K. (2010). Nanoparticles: functionalization and multifunctional applications in biomedical sciences. *Current Medicinal Chemistry*, *17*(36), 4559–4577.

Takahashi, K., Kato, H., Saito, T., Matsuyama, S., & Kinugasa, S. (2008). Precise measurement of the size of nanoparticles by dynamic light scattering with uncertainty analysis. *Particle & Particle Systems Characterization*, *25*(1), 31–38.

Thiruppathi, R., Mishra, S., Ganapathy, M., Padmanabhan, P., & Gulyás, B. (2017). Nanoparticle functionalization and its potentials for molecular imaging. *Advanced Science*, *4*(3), 1600279.

Thomas, M. (2008). *ULTRAVIOLET AND VISIBLE SPECTROSCOPY, (SET PRICE OF 34 BOOKS)*. John Wiley & Sons.

Thompson, D. T. (2007). Using gold nanoparticles for catalysis. *Nano Today*, *2*(4), 40–43. https://doi.org/10.1016/S1748-0132(07)70116-0

Turner.T. (1908). Transparent silver and other metallic films. *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character*, *81*(548), 301–310. https://doi.org/10.1098/rspa.1908.0084

Wadegaonkar, V. P., & Wadegaonkar, P. A. (2013). Withanone as an inhibitor of survivin: A potential drug candidate for cancer therapy. *Journal of Biotechnology*, *168*(2), 229–233.

Werner, P., Eichler, S., Mariani, G., Kögler, R., & Skorupa, W. (1997). Investigation of C x Si defects in C implanted silicon by transmission electron microscopy. *Applied Physics Letters*, *70*(2), 252–254.

Whiteley. C. (2011). *Metal Nanoparticles in Microbiology* (M. Rai & N. Duran, Eds.). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-18312-6

Yang, T., Li, Z., Wang, L., Guo, C., & Sun, Y. (2007). Synthesis, characterization, and self-assembly of protein lysozyme monolayer-stabilized gold nanoparticles. *Langmuir*, *23*(21), 10533–10538.

Yin, L. (2012). Dynamic Light Scattering. *Nanotechnology Research Methods for Foods and Bioproducts*, 145–161. https://doi.org/10.1002/9781118229347.ch8

Yu, Y.-Y., Chang, S.-S., Lee, C.-L., & Wang, C. R. C. (1997). Gold nanorods: electrochemical synthesis and optical properties. *The Journal of Physical Chemistry B*, *101*(34), 6661–6664.

Yuan, Z., Hu, C.-C., Chang, H.-T., & Lu, C. (2016). Gold nanoparticles as sensitive optical probes. *Analyst*, *141*(5), 1611–1626.

Zhao, P., Li, N., & Astruc, D. (2013). State of the art in gold nanoparticle synthesis. *Coordination Chemistry Reviews*, *257*(3–4), 638–665.

Zou, T., Lum, C. T., Lok, C.-N., Zhang, J.-J., & Che, C.-M. (2015). Chemical biology of anticancer gold (III) and gold (I) complexes. *Chemical Society Reviews*, *44*(24), 8786–8801.

zur Hausen, H., & Rösl, F. (1994). Pathogenesis of cancer of the cervix. *Cold Spring Harbor Symposia on Quantitative Biology*, *59*, 623–628.