A DISSERTATION ON

''BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES BY USING *BOSWELLIA SERRATA* GUM RESIN EXTRACT AND INVESTIGATION OF THEIR ANTICANCER EFFICACY AGAINST PROSTATE CANCER PC3 CELLS''

SUBMITTED TO THE
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FOR THE
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IN BIOTECHNOLOGY

BY

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TO WHOM IT MAY CONCERN

This is to certify that **Mr. MOHD AFZAL GOUR** student of M.Sc. Biotechnology (IV semester), Integral University has completed his four months dissertation work entitled **"Biological Synthesis of Silver Nanoparticles by using *Boswellia serrata* Gum Resin Extract and Investigation of their Anticancer Efficacy Against Prostate Cancer PC3 Cells"** successfully. He has completed this work from Feb to June 2022 under the guidance of **Dr. Irfan Ahmad Ansari**. The dissertation was a compulsory part of his M.Sc. degree.

I wish him good luck and bright future.

Dr. Snober S. Mir

Head

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Mr. MOHD AFZAL GOUR** during the months Feb– June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by him are genuine and script of the thesis has been written by the candidate himself. The thesis entitled is "**Biological Synthesis of Silver Nanoparticles by using *Boswellia serrata* Gum Resin Extract and Investigation of their Anticancer Efficacy Against Prostate Cancer PC3 Cells"** is 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, (U.P).

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LIST OF ABBREVIATIONS

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

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INTRODUCTION

INTRODUCTION

Nanobiotechnology has emerged as an intersection of nanotechnology and biotechnology in which tools from nanotechnology are developed and applied to study biological phenomena including nanodevices, nanoparticles (NPs), or other nanostructures possessing at least one dimension sized from 1 to 100 nanometres, leading to various commercial and biomedical applications in many fields, such as electronics, catalysis, agriculture and biomedicine, etc, owing to their unique chemical, physical, electrical and magnetic properties *(Barabadi, 2017).* Nanoparticles (NPs) and nanostructured materials (NSMs) represent an active area of research and a techno-economic sector with full expansion in many application domains. NPs and NSMs have gained prominence in technological advancements due to their tunable physicochemical characteristics such as melting point, wettability, electrical and thermal conductivity, catalytic activity, light absorption and scattering resulting in enhanced performance over their bulk counterparts. A nanometre (nm) is an International System of Units that represents 10^{-9} meter in length. In principle, NMs are described as materials with length of 1–1000 nm in at least one dimension; however, they are commonly defined to be of diameter in the range of 1 to 100 nm (*Boverhof et al., 2015*).

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties (*Gurunathan et al., 2015a; Li et al., 2010; Mukherjee et al., 2001*). Due to their peculiar properties, they have been used for several applications, including as antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopaedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumour-killing effects of anticancer drugs (Chernousova & Epple, 2013).

Boswellia species (Burseraceae), which are trees native to Ethiopia, Somalia, India, and Arabic peninsula, produce a gum resin that is known as olibanum (frankincense). This gum resin has long been used in Ayurvedic and traditional Chinese medicine to treat a

variety of health aspects such as inflammatory and arthritic diseases. The search for the active principles of the resin resulted in the isolation of boswellic acids that belong to ursane and oleanane-type pentacyclic triterpenes. These acids have also been shown to possess potential chemo preventive effects, e.g., they inhibited the growth of brain tumour and meningioma cells as well as they induced apoptosis in human leukaemia (*Ahmed et al., 2015)*.

Cancer results from the outgrowth of a clonal population of cells from tissue. The development of cancer, referred to as carcinogenesis, can be modelled and characterized in a number of ways. One way to describe this process is to illustrate the essential features of both cancer cells and tumours: the “hallmarks” of cancer. Cancer development requires the acquisition of six fundamental properties: self-sufficient proliferation, insensitivity to anti-proliferative signals, evasion of apoptosis, unlimited replicative potential, the maintenance of vascularization, and, for malignancy, tissue invasion and metastasis. Cancer can also be considered with regard to a step-wise development functionally grouped into three phases: initiation, promotion, and progression. Initiation is characterized by genomic changes within the “cancer cell,” such as point mutations, gene deletion and amplification, and chromosomal rearrangements leading to irreversible cellular changes. Tumour development is promoted by the survival and clonal expansion of these “initiated” cells. Progression encompasses a substantial growth in tumour size and either growth-related or mutually exclusive metastasis.(Rakoff-Nahoum, 2006).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Nanotechnology

Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale to investigating whether we can directly control matters on/in the atomic scale/level. This idea entails the application of fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, microfabrication, etc.(*Fakruddin et al., 2012a*).

Nanobiotechnology

Nanobiotechnology is an emerging science concerned with the integration of biological principles with nanotechnology to improve the strategies for nanoparticles synthesis and applications. Various biological routes have been adopted for the synthesis of nanoparticles such as the use of plant extracts, bacteria, fungi, algae, and metabolites of arthropods (*Adelere & Lateef, 2021*). Nanotechnology equipment's are capable of manipulating physical as well as chemical properties of a substance at molecular levels. On the other hand, biotechnology uses the knowledge and techniques of biology to manipulate molecular, genetic and cellular processes to develop products and services and is used in diverse fields from medicine to agriculture. Nanobiotechnology is considered to be the unique fusion of biotechnology and nanotechnology by which classical micro-technology can be merged to a molecular biological approach in real. Through this methodology, atomic or molecular grade machines can be made by mimicking or incorporating biological systems, or by building tiny tools to study or modulate diverse properties of a biological system on molecular basis. Nanobiotechnology may, therefore, ease many avenues of life sciences by integrating cutting-edge applications of information technology & nanotechnology into contemporary biological issues. This technology has potential to remove obvious boundaries between biology, physics and chemistry to some extent, and shape up our current ideas and

understanding. For this reason, many new challenges and directions may also arise in education, research & diagnostics in parallel by the extensive use of nanobiotechnology with the passage of time.(*Fakruddin et al., 2012a*) nanotechnology deals with developing materials, devices, or other structures possessing at least one dimension sized from 1 to 100 nanometers. Meanwhile, Biotechnology deals with metabolic and other physiological processes of biological subjects including microorganisms. Association of these two technologies, i.e., nanobiotechnology can play a vital role in developing and implementing many useful tools in the study of life.

Nanoparticles

The term nano is adapted from the Greek word meaning “dwarf.” When used as a prefix, it implies 10^{-9}. A nanometre (nm) is one billionth of a meter, or roughly the length of three atoms side by side. A DNA molecule is 2.5 nm wide, a protein approximately 50 nm, and a flu virus about 100 nm. A human hair is approximately 10,000 nm thick. A nanoparticle is a microscopic particle with at least one dimension less than 100 nm. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. A bulk material has constant physical properties regardless of its size, but at the nanoscale this is often not the case. Several well characterized bulk materials have been found to possess most interesting properties when studied in the nanoscale. There are many reasons for this including the fact that nanoparticles possess a very high aspect ratio. In the case of silver nanoparticles (AgNPs), this allows them to easily interact with other particles and increases their antibacterial efficiency. This effect is extremely robust, and as little as 1 g of AgNPs is known to impart antibacterial properties to hundreds of square meters of substrate material.*(Thakkar et al., 2010*)

A nanoparticle can be either a zero dimensional where the length, breadth and height is fixed at a single point for example nano dots, one dimensional where it can possess only one parameter for example graphene, two dimensional where it has length and breadth for example carbon nanotubes or three dimensional where it has all the parameters such as length, breadth and height for example gold nanoparticles. The nanoparticles are of different shape, size and structure. It be spherical, cylindrical, tubular, conical, hollow

core, spiral, flat, etc. or irregular and differ from 1 nm to 100 nm in size. The surface can be a uniform or irregular with surface variations. Some nanoparticles are crystalline or amorphous with single or multi crystal solids either loose or agglomerated (*Machado et al., 2015b*).

Synthesis of Nanoparticles

The nanoparticles are synthesised by various methods that are categorised into bottom-up or top-down method. A simplified representation of the process is presented in figure below

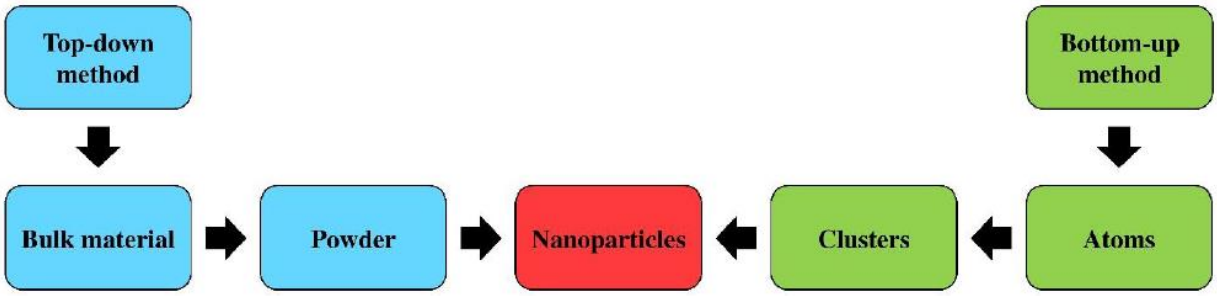


Fig 1- **Synthesis of Nanoparticles** (Ealias & Saravanakumar, 2017a)

1) Bottom-up method

Bottom-up or constructive method is the build-up of material from atom to clusters to nanoparticles. Sol-gel, spinning, chemical vapour deposition (CVD), pyrolysis and biosynthesis are the most commonly used bottom-up methods for nanoparticle production

a) Sol-gel Method

The sol – a colloidal solution of solids suspended in a liquid phase. The gel – a solid macromolecule submerged in a solvent. Sol-gel is the most preferred bottom-up method due to its simplicity and as most of the nanoparticles can be synthesised from this method. It is a wet-chemical process containing a chemical solution acting as a precursor for an

integrated system of discrete particles. Metal oxides and chlorides are the typically used precursors in sol-gel process(*Sivakumar et al., 2013*). The precursor is then dispersed in a host liquid either by shaking, stirring or sonication and the resultant system contains a liquid and a solid phase. A phase separation is carried out to recover the nanoparticles by various methods such as sedimentation, filtration and centrifugation and the moisture is further removed by drying(*Mann et al., 1997*).

b) Spinning.

The synthesis of nanoparticles by spinning is carried out by a spinning disc reactor (SDR). It contains a rotating disc inside a chamber/reactor where the physical parameters such as temperature can be controlled. The reactor is generally filled with nitrogen or other inert gases to
remove oxygen inside and avoid chemical reactions(*Tai et al., 2007a*)

c) Chemical Vapour Deposition (CVD)

Chemical vapour deposition is the deposition of a thin film of gaseous reactants onto a substrate. The deposition is carried out in a reaction chamber at ambient temperature by combining gas molecules. A chemical reaction occurs when a heated substrate comes in contact with the combined gas. This reaction produces a thin film of product on the substrate surface that is recovered and used. Substrate temperature is the influencing factor in CVD*(Bhaviripudi et al., 2007).*

d) Pyrolysis.

Pyrolysis is the most commonly used process in industries for largescale production
of nanoparticle. It involves burning a precursor with flame. The precursor is either liquid or vapour
that is fed into the furnace at high pressure through a small hole where it burns.*(Kammler et al., 2001).*

e) Biosynthesis or Green Synthesis

Biosynthesis is a green and environmentally friendly approach for the synthesis of nanoparticles that are nontoxic and biodegradable(*Kuppusamy et al., 2016*). Biosynthesis uses bacteria, plant extracts, fungi, etc. along with the precursors to produce nanoparticle instead of convention chemicals for bio reduction and capping purposes. (Hasan, 2015). 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*).

2) Top-down method

Top-down or destructive method is the reduction of a bulk material to nanometric scale particles.

Mechanical milling, nanolithography, laser ablation, sputtering and thermal decomposition are some of the most widely used nanoparticle synthesis methods.

a) Mechanical milling.

Among the various top-down methods, mechanical milling is the most extensively used to produce various nanoparticles. The mechanical milling is used for milling and post annealing of nanoparticles during synthesis where different elements are milled in an inert atmosphere.*(Yadav et al., 2012).*

b) Nanolithography

Generally, lithography is the process of printing a required shape or structure on a light sensitive material that selectively removes a portion of material to create the desired

shape and structure. The main advantages of nanolithography are to produce from a single nanoparticle to a cluster with desired shape and size.*(Hulteen et al., 1999*)

c) Laser Ablation

Laser Ablation Synthesis in Solution (LASiS) is a common method for nanoparticle production from various solvents. The irradiation of a metal submerged in a liquid solution by a laser beam condenses a plasma plume that produces nanoparticles.(*Amendola & Meneghetti, 2009*).

d) Sputtering

Sputtering is the deposition of nanoparticles on a surface by ejecting particles from it by colliding with ions*(Shah & Gavrin, 2006).*

e) Thermal decomposition

Thermal decomposition is an endothermic chemical decomposition produced by heat that breaks the chemical bonds in the compound. The specific temperature at which an element chemically decomposes is the decomposition temperature. The nanoparticles are produced by decomposing the metal at specific temperatures undergoing a chemical reaction producing secondary products.*(Salavati-Niasari et al., 2008a)*

Types and classification of nanomaterials

Most current NPs and NSMs can be organized into four material-based categories (the references refer to recent reviews on these different categories of NMs).

1. Carbon-based nanomaterials:

Generally, these NMs contain carbon, and are found in morphologies such as hollow tubes, ellipsoids or spheres. Fullerenes (C60), carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr), and carbon onions are included under the carbon-based NMs category. Laser ablation, arc discharge, and chemical vapor deposition (CVD) are the important production methods for these carbon-based materials fabrication (except carbon black)(“Carbon-Based Nanomaterials,” 2016). The nanoparticles made completely of carbon are knows as carbon based.

I. Fullerenes

Fullerenes (C60) is a carbon molecule that is spherical in shape and made up of carbon atoms held together by sp2 hybridization. About 28 to 1500 carbon atoms forms the spherical structure with diameters up to 8.2 nm for a single layer and 4 to 36 nm for multi-layered fullerenes.

II. Graphene

Graphene is an allotrope of carbon. Graphene is a hexagonal network of honeycomb lattice made up of carbon atoms in a two-dimensional planar surface. Generally, the thickness of the graphene sheet is around 1 nm.

III. Carbon Nano Tubes (CNT)

Carbon Nano Tubes (CNT), a graphene nano foil with a honeycomb lattice of carbon atoms is wound into hollow cylinders to form nanotubes of diameters as low as 0.7 nm for a single layered and 100 nm for multi-layered CNT and length varying from a few micrometres to several millimetres. The ends can either be hollow or closed by a half fullerene molecule.

IV. Carbon Nanofiber.

The same graphene nano foils are used to produce carbon nanofiber as CNT but wound into a cone or cup shape instead of a regular cylindrical tube.

V. Carbon black

An amorphous material made up of carbon, generally spherical in shape with diameters from 20 to 70 nm. The interaction between the particles is so high that they bound in aggregates and around 500 nm agglomerates are formed.(*Ealias & Saravanakumar, 2017b).*

2. Organic nanoparticles

Organic nanoparticles Dendrimers, micelles, liposomes and ferritin, etc. are commonly known the organic nanoparticles or polymers. These nanoparticles are biodegradable, non-toxic, and some particles such as micelles and liposomes have a hollow core (Figure1), also known as Nano capsules and are sensitive to thermal and electromagnetic radiation such as heat and light*(Tiwari et al., 2008)*

These unique characteristics makes them an ideal choice for drug delivery. The drug carrying capacity, its stability and delivery systems, either entrapped drug or adsorbed drug system determines their field of applications and their efficiency apart from their normal characteristics such as the size, composition, surface morphology, etc. The organic nanoparticles are most widely used in the biomedical field for example drug delivery system as they are efficient and also can be injected on specific parts of the body that is also known as targeted drug delivery. Inorganic nanoparticles Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide-based nanoparticles are generally categorised as inorganic nanoparticles

3. Metal based

Nanoparticles that are synthesised from metals to nanometric sizes either by destructive or constructive methods are metal based nanoparticles. Almost all the metals can be synthesised into their nanoparticles*(Salavati-Niasari et al., 2008b*) The commonly used metals for nanoparticle synthesis are aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn). The nanoparticles have distinctive properties such sizes as low as 10 to 100nm, surface characteristics like high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline and amorphous structures, shapes like spherical and cylindrical and colour, reactivity and sensitivity to environmental factors such as air, moisture, heat and sunlight etc.

4. Metal oxides based

The metal oxide-based nanoparticles are synthesised to modify the properties of their respective metal-based nanoparticles, for example nanoparticles of iron (Fe) instantly

oxidises to iron oxide (Fe_2O_3) in the presence of oxygen at room temperature that increases its reactivity compared to iron nanoparticles. Metal oxide nanoparticles are synthesised mainly due to their increased reactivity and efficiency.(*Tai et al., 2007b*) The commonly synthesised are Aluminium oxide (Al_2O_3), Cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite (Fe_3O_4), Silicon dioxide (SiO_2), Titanium oxide (TiO_2), Zinc oxide (ZnO). These nanoparticles have possessed an exceptional property when compared to their metal counterparts.

Nanoparticles Characterization Techniques

UV- VIS Absorption Spectroscopy

UV-Vis Absorption Spectroscopy gives UV absorption of the amorphous gels and crystalline ceramic samples heated at different temperatures.(Srivastava, 2012) 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.

Beer Lambert’s Law states that

$$\mathbf{A = \varepsilon bc}$$

Where ε is a constant of proportionality, called the absorptivity.

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 & Patri, 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, 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.(Babick, 2020). The speed of this Brownian motion is measured and provides the translational diffusion coefficient D. This diffusion coefficient can be converted into a hydrodynamic diameter (D_H) using the Stokes-Einstein equation.

$$\mathbf{DH=kT/3\ \pi\ \eta D}$$

where k is the Boltzmann constant, T is the temperature and η is the dispersant viscosity (Kaszuba *et al*., 2008).

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, 2012b).

Transmission Electron Microscopy

TEM is an imaging technique whereby a beam of photographic film (see electron microscope), or to be detected by a CCDcamera. 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.(Michael J. K. Thomas & David J. Ando, 1996).

FTIR

FTIR can be used to analyse a wide range of materials in bulk or thin films, liquids, solids, pastes, powders, fibres, and other forms. FTIR analysis can give not only qualitative (identification) analysis of materials, but, with relevant standards, can be used for quantitative (amount) analysis. FTIR can be used to analyse samples up to ~11 millimetres in diameter and either measure in bulk or the top ~1 micrometre layer. FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by any extract.(Devaraj *et al*., 2013).

Silver Nanoparticles

Silver nanoparticles (AgNPs) have emerged as a superior product in the field of nanotechnology.1 Nanosilver is one of the nanomaterials with the highest degree of commercialization,1 and silver has gained much interest because of its distinctive physical and chemical properties, including conductivity, chemical stability, and catalytic and antibacterial activities.2–4 Because of their unique properties and high surface area to volume ratio, AgNPs have been used extensively as antibacterial agents in the health industry, food storage,5 textile coating,6 and a number of environmental2,4,7 and biomedical applications, including utility as antiangiogenic8 and anticancer agents.(Gurunathan *et al*., 2015b) Silver nanoparticles are one of the most attractive nanomaterials for commercialization applications. They have been used extensively as electronic products in the industry, anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications. As anti-bacterial agents, silver nanoparticles were used for a wide range of applications from disinfecting medical devices and home appliances to water treatment.(Natsuki, 2015). AgNPs have been acknowledged to be developed for several physical, biological, and pharmaceutical purposes that may be directed by an assortment of techniques, including spark discharging, electrochemical reduction, solution irradiation, and cryochemical synthesis and control approximately 20–15,000 silver atoms. They may be engineered to have different forms, including, fields, particles, rods, square blocks, wires, film and coatings.(Beyene *et al*., 2017a).

Synthesis of silver nanoparticles

A. Physical method

The most important physical approaches are evaporation-condensation and laser ablation]. The absence of solvent contagion in the equipped thin films and the homogeny of NPs distribution are the compensation of physical synthesis methods in contrast with chemical processes. Tube furnace syntheses of silver NPs at atmospheric pressure has some disadvantages such as energy consumption, slow synthesis and call for high concentration. Laser ablation of metallic bulk materials can be synthesized AgNPs in solution. Depends upon various factors, including the wavelength of the laser interrupting

the metallic target, the period of the laser pulses, the ablation time extent and the efficient liquid medium, with or without the existence of surfactants, and the laser power are some of the factors which determine the ablation effectiveness and the characteristics of synthesized nano-silver particles. From available methods, Laser ablation is a unique and significant method which results pure and clean metallic nano-particles without using chemical reagents in solution.(Beyene *et al*., 2017b)

B. Chemical methods

The most common approach for synthesis of silver NPs is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol process, Tollen's reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag^+) in aqueous or non-aqueous solutions. These reducing agents reduce Ag^+ and to the formation of metallic silver (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of metallic colloidal silver particles. It is important to use protective agents to stabilize dispersive NPs during the course of metal nanoparticle preparation, and protect the NPs that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration.(Iravani *et al*., 2014).

C. Biological method

I. Synthesis of Silver Nanoparticles Mediated by Fungi

Fungi have excellent potential for the production of many compounds that can be used in different applications. Around 6,400 bioactive substances are known to be produced by microscopic filamentous fungi (ascomycetes and imperfect fungi) and other fungal species. These organisms are widely used as reducing and stabilizing agents, due to their heavy metal tolerance and capacity to internalize and bioaccumulate metals. Furthermore, fungi can be easily cultivated on a large scale and can produce nanoparticles with controlled size and morphology. Fungi have advantages over other microorganisms, in that they produce large quantities of proteins and enzymes, some of which can be used for the fast and sustainable synthesis of nanoparticles.

The mechanism of biogenic synthesis of nanoparticles using fungi may be intracellular or extracellular. In the case of intracellular synthesis, the metal precursor is added to the mycelial culture and is internalized in the biomass. Consequently, extraction of the nanoparticles is required after the synthesis, employing chemical treatment, centrifugation, and filtration to disrupt the biomass and release the nanoparticles. In extracellular synthesis, the metal precursor is added to the aqueous filtrate containing only the fungal biomolecules, resulting in the formation of free nanoparticles in the dispersion.(Guilger-Casagrande & Lima, 2019).

II. Synthesis by bacteria

Biological synthesis of nanoparticles involves a natural phenomenon occurring in bacterial, fungal, and plant biosystems, thereby generating biocompatible nanomaterials having therapeutic applications. The first report on bacteria-mediated AgNP synthesis came in 1999 when Klaus and co-workers reported the accumulation of AgNPs inside the cells of Pseudomonas stutzeri AG259, a bacterium isolated from a silver mine. The bacteria exhibited the property to survive in an extreme silver-rich environment, which might be the possible explanation for accumulation of nano silver. After that, series of bacteria, both Gram-negative and Gram-positive, have been screened for the synthesis of AgNPs.(Singh *et al*., 2015).

III. Synthesis by plants

Plant-mediated synthesis of AgNPs seems to be very rapid, simple, dependable, non-toxic and eco-friendly. Plant-mediated synthesis of AgNPs enables advancement over chemical and physical methods and easily scaled up for large-scale synthesis. The very first article on the Plant-mediated synthesis of AgNPs using Alfalfa (Medicago sativa) has been reported in 2003 a step toward Plant-mediated nanotechnology. The synthesis of metal nanoparticles using plant extracts deliver beneficial over other biological synthesis methods which are associated with very difficult procedures such as maintaining microbial cultures. After synthesis, AgNPs characterisation is essential to investigate their characteristic features such as surface area, morphology, size, shape, aggregation and solubility, etc.(Rajeshkumar & Bharath, 2017).

Boswellia serrata

Boswellia serrata (family Burseraceae) is an oleo-gum-resin found in dry hilly parts of India. It is a large branching medium size tree known as 'Dhup', Indian frankincense or Indian Olibanum. *Boswellia serrata (B*. serrata) has been used for a variety of therapeutic purposes such as cancer, inflammation, arthritis, asthma, psoriasis, colitis and hyperlipidemia . The essential oil of B. serrata is a mixture of mono, di and sesquiterpenes whereas gum portion consists of pentose and hexose sugar with oxidizing and digestive enzymes (Sharma *et al*., 2010). Gum-resin extracts of *Boswellia serrata* have been traditionally used in folk medicine for centuries to treat various chronic inflammatory diseases. The resinous part of *Boswellia serrata* possesses monoterpenes, diterpenes, triterpenes, tetracyclic triterpene acids and four major pentacyclic triterpenic acids i.e., β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid, responsible for inhibition of pro-inflammatory enzymes. Out of these four Boswellia acids, acetyl-11-keto-β-Boswellia acid is the most potent inhibitor of 5-lipoxygenase, an enzyme responsible for inflammation.(Siddiqui, 2011).

Application of AgNPs

a. Anticancer activity of AgNPs

AgNPs have been observed to exhibit good anticancer activities in breast cancer, cervical cancer, colon cancer, ovarian cancer, pancreatic ductal adenocarcinoma, lung cancer, hepatocellular carcinoma, melanoma, osteosarcoma, etc. Several studies confirm that the anticancer activities of AgNPs with various sizes, shapes and doses/concentrations are discrepant in different cancer cells.

b. Antidiabetic Agent

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia. DM is due to either insufficient insulin secretion or insulin resistance of the cell. Commonly used hypoglycemic agents can lower blood sugar by promoting secretion of insulin or increasing cell sensitivity. In recent studies, it is noticed that AgNPs synthesized by plant

extracts exhibit antidiabetic potential. Arumugam *et al*. synthesized AgNPs using leaf extract of Solanum nigrum and evaluated the anti-hyperglycemic effect in alloxan-induced diabetic rats. They found that the blood glucose level of diabetic rats decreased when treated with AgNPs for 14 days and 21 days without significant acute toxicity.

c. Biosensing and Imaging

Surface-enhanced Raman scattering (SERS) has attracted the attention of noble metals with Raman signals in many application strategies, including biochemical sensing, analytical chemistry, and materials science [303]. Among these nanomaterials, AgNPs can be used as a cost-effective surface-enhanced Raman scattering substrate. Nanoparticles containing AgNPs can be used as biosensors to detect blood glucose, enzymes, molecular markers of tumor cells, pathogens, etc. (Xu *et al*., 2020)

d. Antiviral Activity

In modern human history, viruses have been found to be one of the most terrible human diseases
pathogens. Despite the apparent structural simplicity, viruses reveal a huge threat in the face of
dangerous diseases—Spanish influenza, HIV, Ebola, and Marburg, and finally, the 2020 pandemic
caused by COVID-19—proving to us how little we know about fighting viruses. The pathogenic nature of viruses consists of attachment and penetration into the host cell. In this case, the virus binds to ligands and proteins on the cell membrane surface using its own protein components.
Preventing such binding appears to be the best way of avoiding cell infection. AgNPs bind to the protective coat of the protein of the virus, suppressing attachment; and AgNPs bind to the virus DNA or RNA, suppressing replication or virus proliferation inside host cells.

For example, AgNPs have been shown to inhibit the initiation of transmitted gastroenteritis virus (TGEV) infection by binding to a surface protein, S-glycoprotein. It has been suggested that silver nanoparticles can change the structure of surface proteins, thereby reducing their recognition and adhesion to the host receptor.

e. Antioxidant Activity

Reactive oxygen species (ROS) such as hydroxyl, epoxyl, superoxide, peroxylnitrile, and singlet
oxygen generate oxidative stress, leading to the growth of various diseases such as inflammation,
atherosclerosis, aging, cancer, and neurodegenerative disorders. The antioxidant properties of a silver phyto-nanosystem make them useful in the treatment of disease. Thus, silver phyto-nanoparticles obtained from extracts of ornamental flower plants Hyacinthus orientalis and Dianthus caryophyllus (oriental hyacinth and garden clove) were found to have high antioxidant activity.(Mikhailova, 2020).

Application of Nanotechnology

1. Drug Delivery:

Nanoparticles as therapeutics can be delivered to targeted sites, including locations that cannot be easily reached by standard drugs. For instance, if a therapeutic can be chemically attached to a nanoparticle, it can then be guided to the site of the disease or infection by radio or magnetic signals. These nanodrugs can also be designed to "release" only at times when specific molecules are present or when external triggers (such as infrared heat) are provided. At the same time, harmful side effects from potent medications can be avoided by reducing the effective dosage needed to treat the patient. By encapsulating drugs in nanosized materials (such as organic dendrimers, hollow polymer capsules, and nano shells), release can be controlled much more precisely than ever before.(LaVan *et al*., 2002)

2. Gene delivery

Nanotechnological tools in human gene therapy have been tested and nanoparticle-based nonviral vectors (usually 50-500 nm in size) in transportation of plasmid DNA described. Therefore, successful introduction of less immunogenic nanosized gene carriers as a substitution of the disputed viral vectors seems beneficial in repairing or replacing impaired genes in human(Davis, 1997).

3. Nanotechnology in cardiac therapy

Miniaturized nanoscale sensors like quantum dots (QDs), nanocrystals, and nano barcodes are capable of sensing and monitoring complex immune signals in response to cardiac or inflammatory events (Guccione *et al*., 2004a).

4. Liposomes

A liposome being composed of a lipid bilayer can be used in gene therapy due to its ability to pass through lipid bilayers and cell membranes of the target. Recent use of several groups of liposomes in a local delivery has been found to be convincingly effective(Ewert *et al*., 2005; Hart, 2005).

5. Detection:

Many currently used/conventional clinical tests reveal the presence of a molecule or a disease-causing organism by detecting the binding of a specific antibody to the disease-related target. Traditionally, such tests are performed by conjugating the antibodies with inorganic/organic dyes and visualizing the signals within the samples through fluorescence microscopy or electronic microscopy. However, dyes often limit the specificity and practicality of the detection methods. Nanobiotechnology offers a solution by using semiconductor nanocrystals (also referred to as "quantum dots"). These minuscule probes can withstand significantly more cycles of excitations and light emissions than typical organic molecules, which more readily decompose(Drexler, 1992).

6. Nanotechnology as a tool in imaging

Intracellular imaging can be made possible through labelling of target molecules with quantum dots (QDs) or synthetic chromophores, such as fluorescent proteins that will facilitate direct investigation of intracellular signalling complex by optical techniques, i. e. confocal fluorescence microscopy or correlation imaging (Guccione *et al*., 2004b; Lin & Datar, 2006).

7. Individual target probes

Despite the advantages of magnetic detections, optical and colorimetric detections will continue to be chosen by the medical community. Nanosphere (Northbrook, Illinois) is one of the companies that developed techniques that allow/allowing doctors to optically detect the genetic compositions of biological specimens. Nano gold particles studded with short segments of DNA form the basis of the easy-to-read test for the presence of any given genetic sequence. If the sequence of interest in the samples, it binds to complementary DNA tentacles on multiple nanospheres and forms a dense web of visible gold balls. This technology allows/facilitates the detection of pathogenic organisms and has shown promising results in the detection of anthrax, giving much higher sensitivity than tests that are currently being used (Fakruddin *et al*., 2012b).

OBJECTIVE

Objectives

- To biosynthesize silver nanoparticles by using *Boswellia serrata* gum resin extract.
- To characterize silver nanoparticles by using UV- Visible Spectroscopy, Dynamic Light Scattering (DLS) and Zeta potential.
- To check the efficacy of Silver Nanoparticles against prostate cancer (PC3) cells.

MATERIAL AND METHOD

Material and Methods:

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

Plant Collection and extract preparation:

Gum resins of *Boswellia serrata* was procured from Dr. AK Jain, BAMS, Lucknow and authenticated by Dr. Mqbool Ahmad Khan, Deputy Director, CCRUM, Kursi Road, Basaha, Lucknow.

Soxhlet extraction:

Gum resin of Boswellia Serrata were crushed to the the powder form. The powder were than subjected to conventional solvent based soxhlet extraction by using ethanol. The gum resin powder (20gm) were extracted with 250 ml of absolute ethanol for 7 hours at 70 degree temperature until the solvent become colorless. The solvent were then evaporated till dryness under reduced pressure and dried extract were then weighed and stored at -20 degree temperature for further use.

Boswellia serrata mediated synthesis of silver nanoparticles

In vitro synthesis of AgNPs was performed by 3 ml of the prepared plant extract was taken in 20 ml of centrifuge tube and 3mM of silver nitrate salt was added to the plant extract and volume makeup with water. Keep the reaction tube in incubator at about 37°C. After 120 hrs (5 days) the different period the sample was ejected and analysed on a bio spectrum-Kinetic spectrophotometer using a quartz cuvette having the path length of 1 cm to a affirm the synthesis *Boswellia serrata* encapsulated silver nanoparticle subsequently, the solution was filtered using a syringe with a filter having the pore size of 2 micrometre, the unbound proteins and phytochemicals where expelled using ethanol treatment for 30 minutes and utilized further characterization.

Characterization of silver nanoparticles

The transformation of silver salt into silver nanoparticles was investigated by using the shimadz UV-1601 dual beam spectrometer. This measurement has a special resolution of one nanometre (200 nm to 800 nm). The technique is done on the basis of reducing metal salts to synthesize silver nanoparticles result in colour change. Particle size analyser (Zetasizer Nano-ZS, Model ZEN3600, Malvern Instrument Ltd., Malvern, UK) was used to analyse the mean particle size of AgNPs. 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 prostate cancer cell line (PC3) was purchased from National Centre for Cell Science (NCCS), Pune, India. The aforementioned *in-vitro* cytotoxic potential analysis of BS-extract and BS-AgNPs was performed on PC3 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 PC3 cells

PC3 cells were pre-treated with different concentrations of each, BS- Extract, BS-AgNPs incubated for 24 h at 37°C in an atmosphere 5% CO_2. Post-incubation, the morphological changes in PC3 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 BS-extract and BS-AgNPs, PC3 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 BS-extract, BS-AgNPs 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, 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

Boswellia serrata Mediated synthesis of AgNPs (BS-AgNPs)

This study used *Boswellia serrata* gum resin extract as a reducing sugar and capping agent, whereas 3Mm silver nitrate ($AgNO_3$) served as the silver precursor. The synthesis of BS-AgNPs is considered to be induced by the aqueous extracts reducing enzymes and capping agents, such as secondary metabolites. The creation of BS-AgNPs was confirmed visually by a shift in the color of the extract from green to dark brown, indicating silver reduction.

Characterization of BS-AgNPs

The Phyto constitution in *Boswellia serrata* gum resin extract reduced the silver salt ($AgNO_3$) into AgNPs and encapsulated the silver nanoparticle preventing the nanoparticles from the aggregating and providing stability to the BS-AgNPs. The change in colour from light green to dark brown indicated the successful synthesis of BS-AgNPs, and the result of SPR band confirm that at 418 and however there was no discernible peek for *Boswellia serrata* extract. The technique of dynamic light extracting (DLS) was used to determine the average particle size and provide of the particle size distribution of BS-Ag had an average particle size of 65 d nm as shown in figure. Furthermore, the Zeta potential of the prepared BS-AgNPs was observed at the room temperature, to be a -18.6 mV, indicating the significantly high stability of the nanoparticles. When the aqueous dispersion of AgNPs 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.

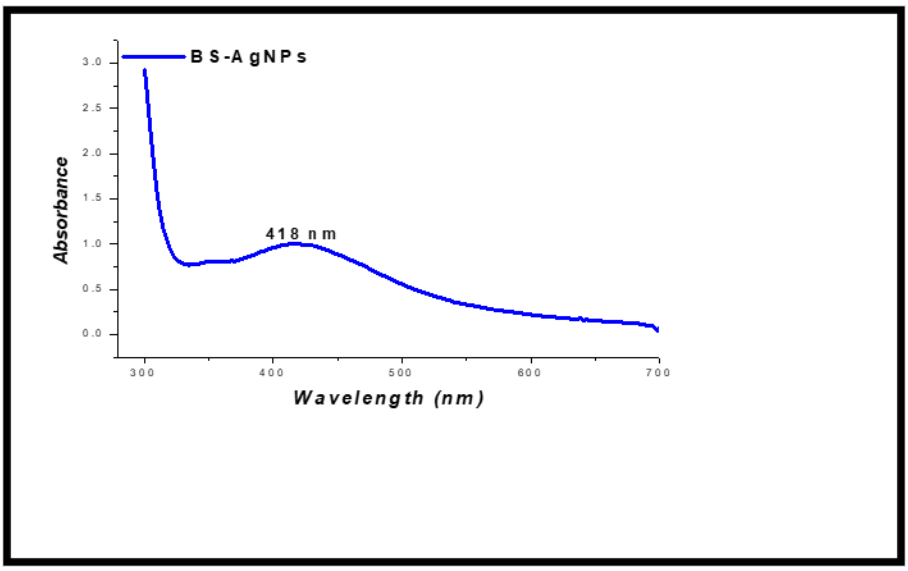


Fig 2: Characterisation of BS-AgNPs under UV-Visible spectra (418 nm).

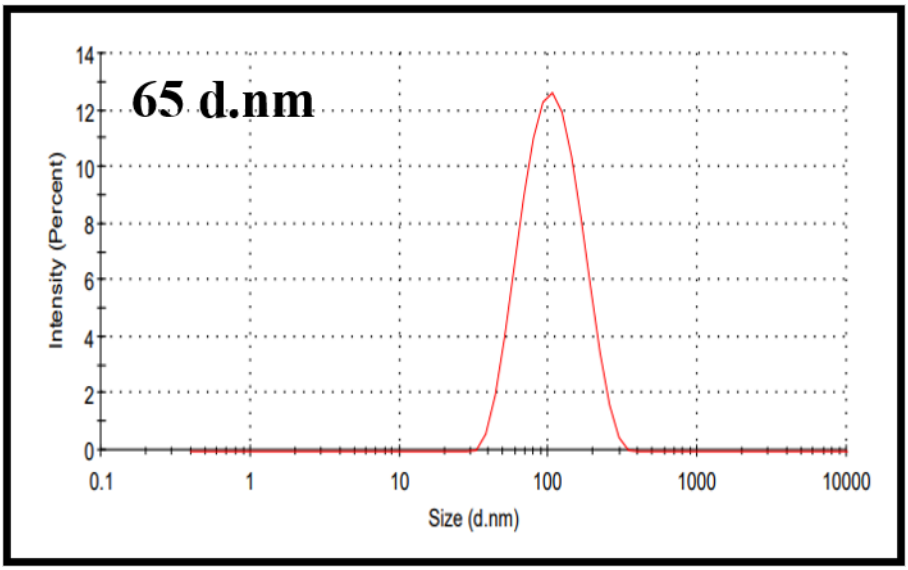


Fig 3: DLS profile of BS-AgNPs showing size of 65 d.nm.

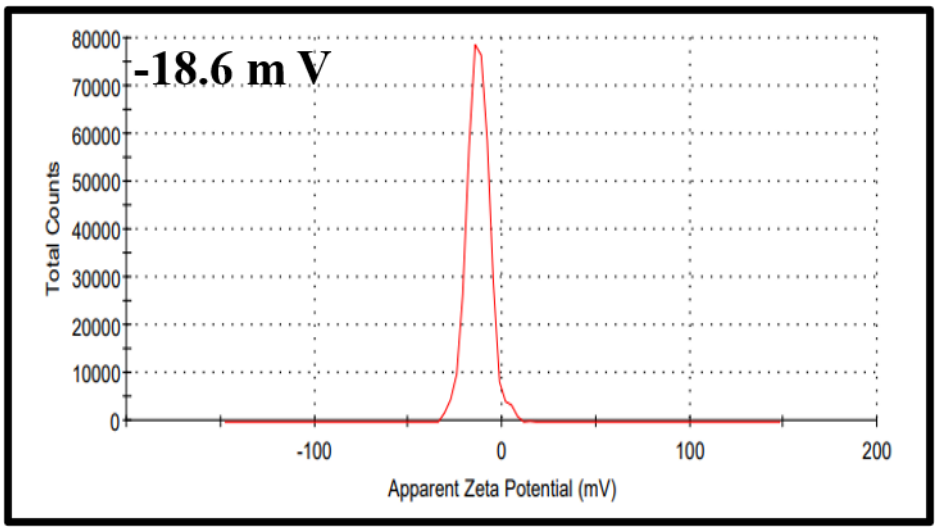


Fig 4: Zeta potential of BS-AgNPs confirmed the stability at -18.6 mV.

Determination of cytomorphological changes in the PC3 cells

Morphological analysis of the BS- extract and BS-AgNPs, treated PC3 cells was performed using a phase contrast microscope. A dose dependent change in the cell morphology was observed in PC3 cells after treatment with BS- extract (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml) and BS-AgNPs, (20 µg/ml, 40 µg/ml, 80 µg/ml) concentrations for 24 h. In the presence of different doses BS- extract and BS-AgNPs, PC3 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 PC3 cells. These morphological changes in Prostate cancer were more evident with the increase in the dose in AgNPs. In contrast, well spread flattened morphology was observed in untreated control cells.

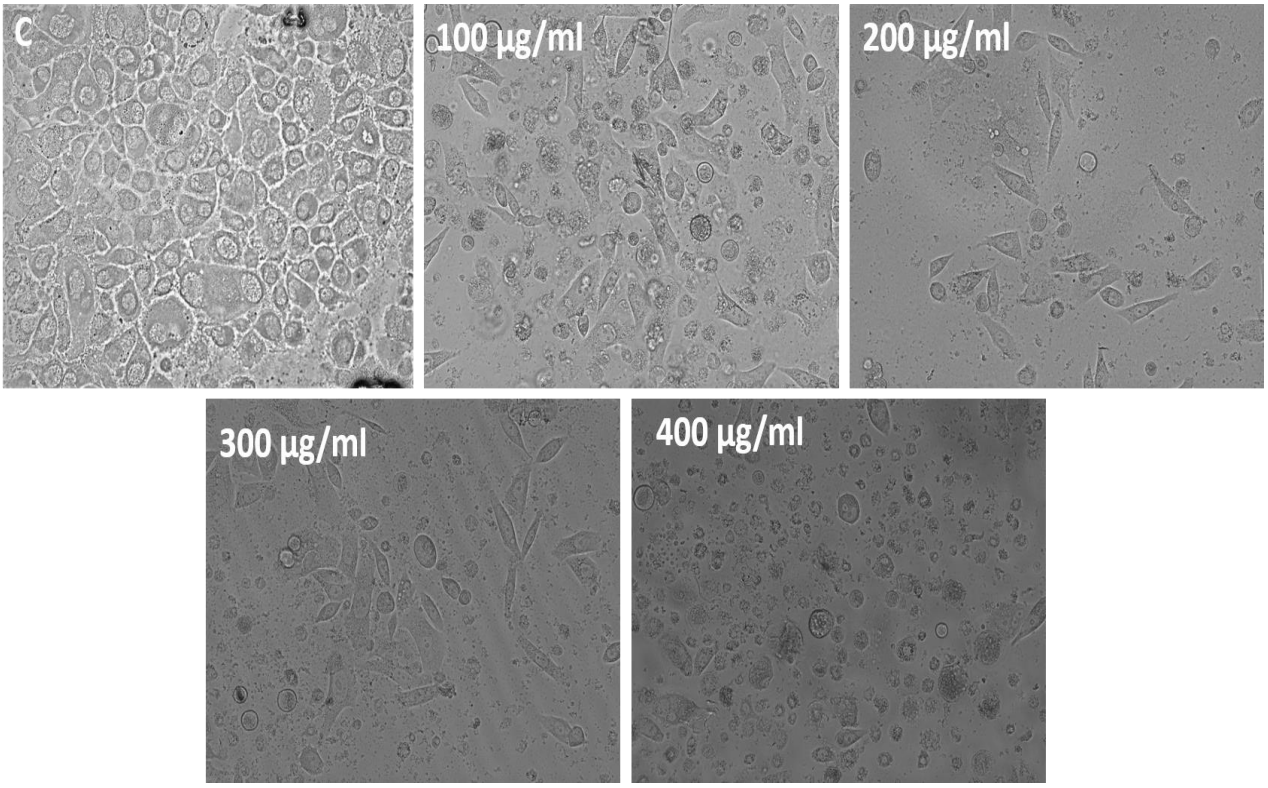


Figure 5-Phase contrast micrographs of prostate cancer cells treated with either vehicle control or different concentration (100 µg/ml,200 µg/ml,300 µg/ml and 400 µg/ml) of BS extract for 24 h in a time and dose-dependent manner. Image shown are representative of three independent experiment (Scale bar:100µm; magnification 20X .

The images of the untreated and treated Prostate cancer cell line (PC3 cells) showed observable morphological changes under phase contrast microscope. The untreated cells revealed progressive cell growth with intact cell morphology under microscope (figure 5). However, severe morphological alterations were noticed in *Boswellia serrata* extract treated PC3 cells in a dose dependent manner (100 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml). Moreover, an increase in detachment and cytoplasmic shrinkage of cells were observed in *Boswellia serrata* extract treated prostate cancer cells which resulted into

greater number of floating cells. Thus, the results confirm that extract *Boswellia serrata* induce cytotoxicity in prostate cancer PC3 cells.

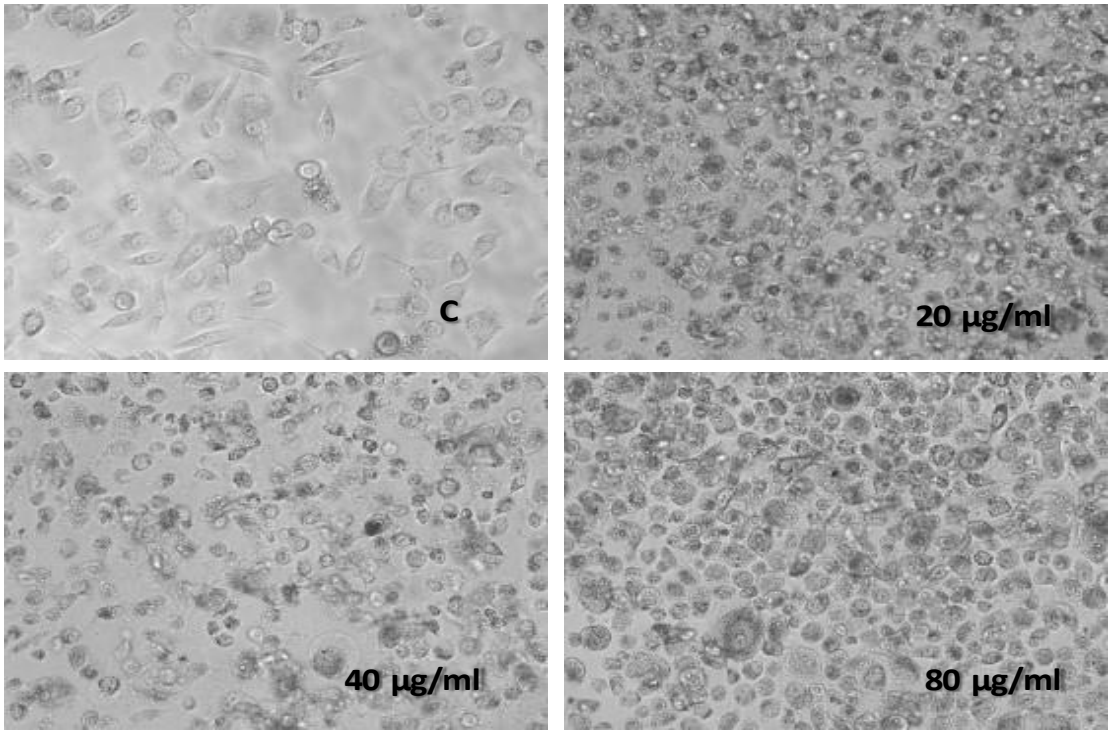


Figure 6*:* Phase contrast micrographs of prostate cancer cells treated with either vehicle control or different concentration (20µg/ml, 40µg/ml, 80 µg/ml) of BS-AgNPs for 24 h in a time and dose-dependent manner. Image shown are representative of three independent experiment (Scale bar:100µm; magnification 20X)

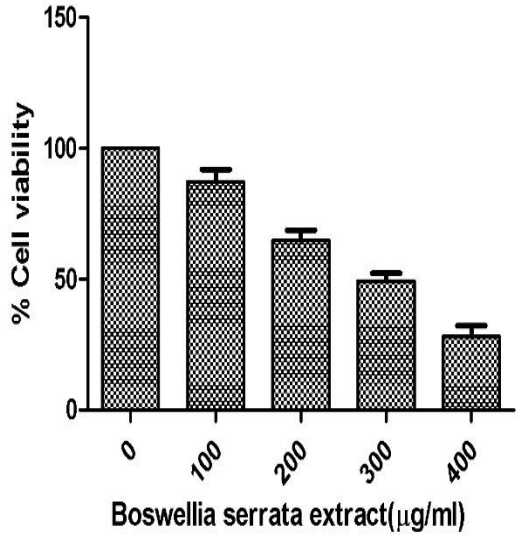
In vitro cytotoxicity of BS-extract and BS-AGNPs

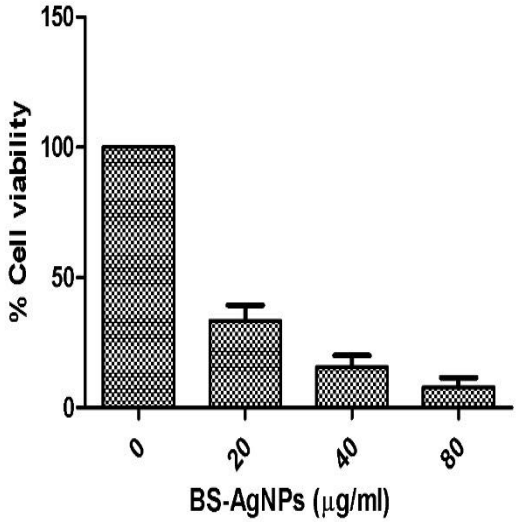
To evaluate the sensitivity of prostate cancer cells to these drugs, PC3 cells were treated with different doses of BS- extract and BS-AgNPs, for 24 h followed by MTT assay. Our results showed that, after 24 h of treatment, AgNPs at IC50=142.40±1.13µg/ml reduced growth of PC3 cells by 50%, while inhibition of 50% viability of PC3 cells was observed at IC50=26±1.19µg/ml of BS-AgNPs, respectively,therefore .AgNPs were found to be

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

In vitro cytotoxicity of BS- extract and BS-AgNPs,

To evaluate the sensitivity of prostate cancer cells to these drugs, PC3 cells were treated with different doses of BS- extract and BS-AgNPs, for 24 h followed by MTT assay. Our results showed that, after 24 h of treatment, BS-extract at a concentration of 195.9±1.07 µg/ml reduced growth of PC3 cells by 50%, while inhibition of 50% viability of PC3 cells was observed at 13.08±26 µg/ml, BS-AgNPs, respectively. BS-AgNPs, were found to be more cytotoxic for prostate cancer cells in comparison to pure extract and the effect was observed to be dose-and time-dependent.





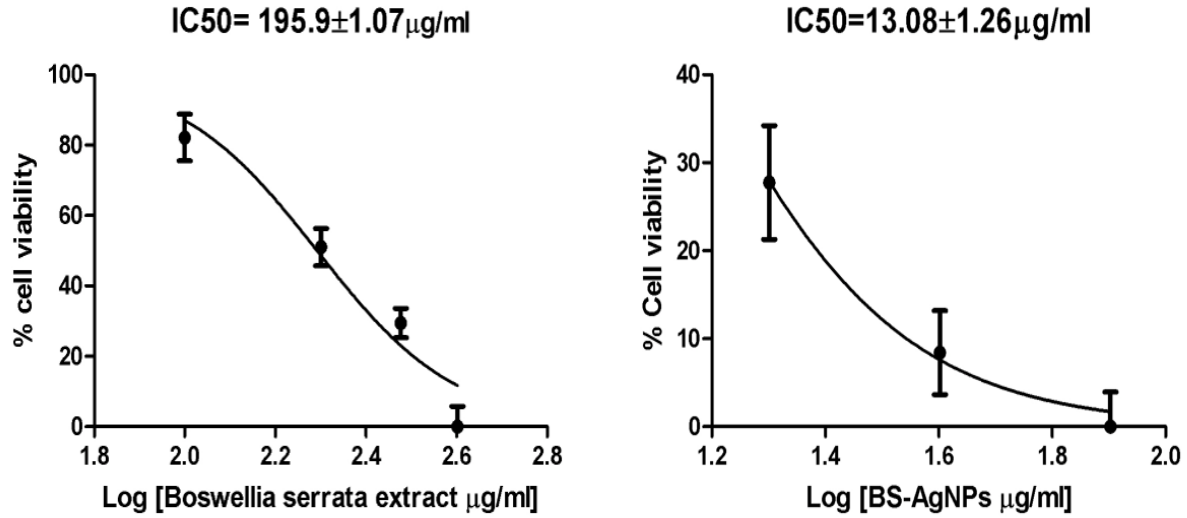


Fig 7- Percent cell viability of PC3 cells treated with different doses of *Boswellia serata* (100-400µg/ml) assessed by MTT Assay 24h. Graph showed that of *Boswellia serata* extract exhibited IC50 value 195.9µg/ml at 24 h against PC3 prostate cancer cell. Percent cell viability of PC3 cells treated with different doses BS-AgNPs (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 BS-AgNPs exhibited IC50 value 13.08±µg/ml at 24 h, against PC3 prostate cancer cell. The result represented are the mean ±SEM of three independent experiment performed in triplicate.

CONCLUSION

Conclusion and future Perspectives:

In this study a gum resin extract mediated green synthesis of silver nanoparticles from *Boswellia serrata* plant and their characterization, anticancer and antioxidant property analysis. This study investigates an efficient and sustainable route of AgNP preparation from 3mM aqueous AgNO3 using gum resin extracts of *Boswellia serrata* plants. The AgNPs were characterized by UV-visible spectrophotometer, particle size analyzer (DLS) and Zeta potential.

This review comprehensively addressed synthesis, characterization, and bio-applications of silver nanoparticles, with special emphasis on anticancer and antioxidant activity and also therapeutic approaches for cancer using AgNPs. Recently, both academic and industrial research has explored the possibility of using AgNPs as a next-generation anticancer therapeutic agent, due to the conventional side effects of chemo- and radiation therapy. Although AgNPs 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 AgNPs 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 AgNPs 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 AgNPs. 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 AgNPs. Eventually, to ensure the biosafety of the use of AgNPs in humans, studies dealing with biocompatibility of AgNPs and their interaction with cells and tissues are inevitable. Finally, the great concern is that the developing nanotechnology-based therapy should be better than available technologies, and it should overcome the limitations of existing treatment techniques. Finally, it has to provide a safe, reliable, and viable treatment of diseases with high accuracy in a patient-friendly manner.

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