A DISSERTATION ON

GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING CATHARANTHUS ROSEUS LEAF EXTRACT AND EVALUATION OF THEIR CYTOTOXIC EFFECT AGAINST CERVICAL CANCER HeLa CELLS.

SUBMITTED TO THE
DEPARTMENT OF BIOSCIENCES
INTEGRAL UNIVERSITY, LUCKNOW

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

BY

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M. Sc. Biotechnology (IV semester)
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UNDER THE SUPERVISION OF

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Sheikh Muskan** student of M.Sc. Biotechnology (IVsemester), Integral University has completed her four months dissertation work entitled "**Green synthesis of silver nanoparticles by using *Catharanthus roseus* leaf extract and evaluation of their cytotoxic effect against cervical cancer HeLa cells.**" successfully. She has completed this work from Feb-June 2022 under the guidance of **Dr. Irfan Ahmad Ansari**. The dissertation was a compulsory part of her M. Sc. degree.

I wish her good luck and bright future.

Dr. Snober S. Mir

Head

Department of Biosciences

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Sheikh Muskan** during the months Feb – June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by the candidate herself. The thesis entitled is "**Green synthesis of silver nanoparticles by using *Catharanthus roseus* leaf extract and evaluation of their cytotoxic effect against cervical cancer HeLa cells."** is therefore, being forwarded for the acceptance in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow (U.P).

Dr. Irfan Ahmad Ansari

Associate Professor

(Department Of Biosciences)

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Sheikh Muskan

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LIST OF ABBREVIATIONS

AgNPs	Silver Nanoparticles
UV-Vis	Ultraviolet Visible
DLS	Dynamic Light Scattering
PLA	Polylactides
PMMA	Poly methyl methacrylate
PEG	Polyethylene glycol
NaBH4	Sodium borohydride
TIAs	Terpenoids indole alkaloids
SPR	Surface plasmon resonance
WHO	World health organization
CNS	Central nervous system
FA-AgNPs	Folic acid coated silver nanoparticles
DOX	Doxorubicin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
FRAP	Ferric reducing antioxidant power

INTRODUCTION

Catharanthus roseus is an evergreen sub herb or herbaceous plant. A native to Madagascar, this herbaceous plant grows to 80 cm to 1 m high and blooms continuously year-round with pink, purple, or white flowers (Hogan, 2003). It is an important medicinal plant of Apocynaceae containing abundant useful alkaloids, used in diabetes, blood pressure, asthma, constipation, cancer and menstrual problems. Vinblastine and vincristine are two powerful anticancer natural products, belonging to the group of terpenoid-indole alkaloids isolated from the pan tropical plant *Catharanthus roseus,* in which they are present only as minor constituents of the complex mixture of about 130 alkaloids produced by this plant. During the last 40 years vinblastine and vincristine have been used for the treatment and cure of thousands of patients, both because of their unique mode of action and their effectiveness..

Catharanthus roseus is native to the Indian Ocean Island of Madagascar. In many tropical and subtropical regions worldwide it has been introduced as a popular ornamental plant. It commercially cultivated in Spain, United States, China, Africa, Australia, India, and Southern Europe for its medicinal uses (PROTA Foundation, Wageningen, The Netherlands, 2011, Łata B., G. Don Phytochemistry Reviews. 2007).

Fig.1 *Catharanthus roseus* (Madagascar periwinkle or Sadabahar)

The Periwinkle is a perennial plant that is very prevalent in areas that are tropical to sub-tropical. However, in areas that are more moderate, the periwinkle is considered an annual, due to the frosty conditions of the fall. It is also commonly found in the southern parts of the United States in California, Texas, Louisiana, Mississippi, Florida, Georgia, and South and North Carolina (Baskaran, *et al.* 2001). Alkaloids are the most potentially active chemical constituents of *Catharanthus roseus*. More than 400 alkaloids are present in the plant, which are used as pharmaceuticals, agrochemicals, flavor and fragrance, ingredients, food additives and pesticides. Study of periwinkle has increased because of its ability to produce secondary metabolites such as terpenoid indole alkaloids that may be used to treat cardiac diseases and certain tumors in mammals (Favali *et al.,* 2004). *Catharanthus roseus (C. roseus)* is an important medicinal plant distributed in many countries. It has attracted increasing attention due to presence of phytochemicals with various biological activities such as antioxidant, antibacterial, antifungal, antidiabetic and anticancer properties. Remarkably, vinblastine and vincristine isolated from this plant were the first plant-derived anticancer agents deployed for clinical use. More scientific evidence has proved the potential health benefits of individual phytochemicals extracted from this plant. Of note, vinblastine and vincristine from *C. roseus*, and their synthetic analogues, have been used in combination with other cancer chemotherapeutic drugs for treating advanced testicular cancer, breast and lung cancers (Cragg *et.al.,* 2005). The drying and extraction process are crucial steps prior to isolation and purification of bioactive compounds from plant material. Therefore, selecting a suitable drying method is very important to maintain the high yield of bioactive compounds and antioxidant power within plant material; however, the economic aspect and the accessibility of drying equipment should also be considered. Similarly, the extraction process is also a key step to obtain bioactive compounds from plant materials. Extraction techniques have been reported to significantly affect the extraction efficiency and stability of bioactive compounds (Goli *et.al*.,2005, Nguyen *et.al*.,2015, Mehmood *et.al*.,2019, Uribe, *et.al.,*2015). The Soxhlet extraction was used to prepare the C. roseus leaves extract for isolation of vindoline, ajmalicine, serpentine and catharanthine (Uniyal, *et.al.,* 2001). Although this method is simple, well established and inexpensive, it is also time consuming and requires large quantities of solvent. Further, it occurs at the boiling point of solvents over a long period that may cause the thermal degradation of bioactive compounds (Wang, *et.al*., 2006).

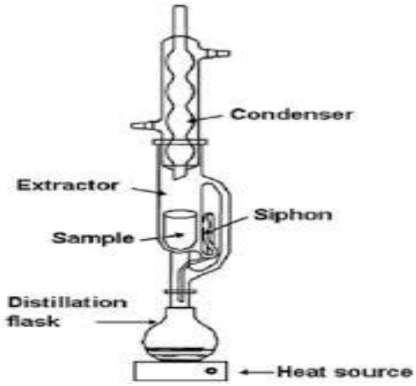


Fig.2 Soxhlet extraction apparatus

Nanotechnology is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale (Mansoori, *et.al.,* 2005). It is an interdisciplinary research field involving biology, medicine and molecular engineering. Its aim is the production of biocompatible and environmentally safe nanoparticles for medical applications using green synthesis methodologies (Mansoori *et.al*., 2007). Nanoparticles are viewed as the fundamental building blocks of nanotechnology (Singh *et.al.,* 2016). They are the starting points for preparing many nanostructured materials and devices.

Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. AgNPs play an important role in nanoscience and nanotechnology, particularly in nanomedicine. 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, orthopedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs (Chernousova *et.al* 2013).

In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous (Gurunathan *et.al.* 2015, Gurunathan *et.al.,* 2009). Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability (Gurunathan *et.al.,*2015). Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs shows much promise.

After synthesis, precise particle characterization is necessary, because the physicochemical properties of a particle could have a significant impact on their biological properties. In order to address the safety issue to use the full potential of any nano material in the purpose of human welfare, in nanomedicines, or in the health care industry, etc., it is necessary to characterize the prepared nanoparticles before application (Lin *et.al*.,2014). To evaluate the synthesized nanomaterials, many analytical techniques have been used, including ultraviolet visible spectroscopy (UV-vis spectroscopy), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), Zeta Potential and so on (Gurunathan S *et.al*.,2015, Sapsford K.E *et.al*.,2011).

Previous studies have documented the cytotoxicity effects of AgNPs on various cancer cell lines with promising results. For example, silver nanoparticles have been proven to be biocompatible to healthy cells and has inhibitory effects on the proliferation of various cancer cell lines including human glioblastoma cells, Dalton's lymphoma ascites (DLA) cell, human cervical carcinoma cell lines (Karunamoorthy *et al.,* 2014), breast carcinoma MCF-7 cell lines, HeLa cell lines (Panchanathan *et al.,* 2013), MDA-MB231 cell lines and human lung cancer A549 cell lines. These preliminary data suggests that AgNPs have great potentials to be applied as anticancer agents.

REVIEW OF LITERATURE:

Nanotechnology

Nanotechnology is a new way of using and manipulating the matter at the molecular scale, whose functional organization is measured in nanometers. Nanotechnology provides a tool for structural analysis at the most important dimensions of organizational structure, atomic and cellular levels, and designs and manufactures synthetic biomaterials on a nanoscale with new treatments and alternative materials emerging.

Nanotechnology, a combination of principles involving biology, physical and chemical that creates nano-sized particles holding particular functions. For this purpose, noble metal nanoparticles like silver, gold, platinum, palladium etc. and non-metallic, inorganic oxides like the zinc oxide, titanium oxide have been widely exploited because of their unique electronic, mechanical, optical, chemical and magnetic properties . The nanoparticles have unique properties of exhibiting larger surface area to volume ratio, size, shape like spherical or rod, etc. due to which they are being used in the various fields of diagnostic biological probes, optoelectronics, display instruments, catalysis, fabricating biological sensors, diagnosis or monitoring diseases like cancer cells, drug discovery, detecting environmental toxic metals or reagents and in therapeutic applications (T.A Davis *et.al*.,2003).

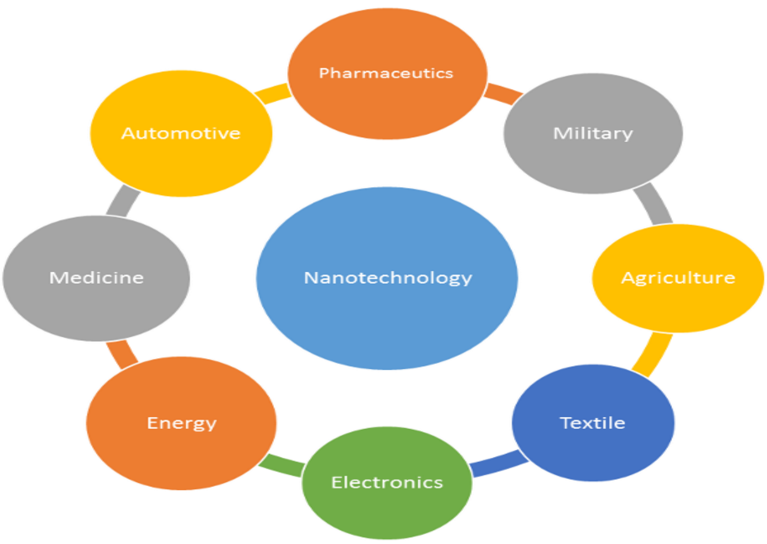


Fig.3 :Nanotechnology application and global market.

NANOPARTICLES: NPs have special enhanced physical and chemical properties compared to their corresponding bulk materials. These properties include a high surface area-to-volume ratio and a unique quantum size effect due to specific electronic structures. In addition to their composition, the properties of NPs depend on their size and shape. Generally, in order to obtain monodispersed NPs and facilitate their internalization by cells, it is necessary to control their size and shape and thus minimize aggregation (Frattini *et.al.,* 2005).

TYPES OF NANOPARTICLES

Regarding their chemical compounds, NPs can be divided into three main groups: organic nanoparticles (liposomes and polymers), inorganic nanoparticles (metals, metal oxide, ceramic, and quantum dots), and carbon-based nanoparticles (Biazar *et.al.,* 2011).

1. LIPOSOMES NANOPARTICLES- These are spherical vesicles with a membrane composed of a lipid bilayer containing an aqueous substance. Liposomes can be synthetized by sonicating a dispersion of amphipatic lipids, such as phospholipids, in water. Liposomes are mainly used for delivering chemotherapeutic drugs in cancer treatment (Ahmad *et.al.,* 2003). They can also incorporate a high number of bioactive materials, including pharmaceutical drugs or food ingredients. Liposomes have great potential applications in nanomedicine, as well as in the food and cosmetics industries, due to their high biocompatibility and biodegradability.

2. POLYMERIC NANOPARTICLE

Most polymeric nanoparticles are known for their biodegradability and biocompatibility, constituting the most commonly used NPs in drug delivery systems. This type of nanoparticle can be made from natural polymers, such as chitosan, or synthetic polymers, such as polylactides (PLA), poly (methyl methacrylate) (PMMA), or polyethylene glycol (PEG).

3. CARBON BASED NANOPARTICLES-These include fullerenes and nanotubes. Fullerenes are novel carbon allotropes with a polygonal structure made up exclusively of 60 carbon atoms. Carbon nanotubes are normally manufactured from chemical vapor deposition of graphite. Carbon-based nanoparticles are considered of interest in biomedical applications due to their physical properties, including high electrical conductivity and excellent mechanical strength, but they are not biodegradable and require surface modifications, as they have a strong tendency to form large aggregates (Vardharajula *et.al.,* 2012).

4. METALLIC NANOPARTICLES- These include precious metals (gold or silver) and magnetic metals (iron oxide or cobalt and manganese doped ferrites).

Silver nanoparticles:

AgNPs exhibit a consistent amount of flexible properties which endorse them for a larger spectrum of applications in biomedicine and related fields. Over the years, silver nanoparticles have been subjected to numerous in vitro and in vivo tests to provide information about their toxic behavior towards living tissues and organisms. AgNPs have high antimicrobial efficacy against many bacteria species including Escherichia coli, Neisseria gonorrhea, Chlamydia trachomatis and also viruses. Due to their novel properties, the incorporation of silver nanoparticles into different materials like textile fibers and wound dressings can extend their utility on the biomedical field while inhibiting infections and biofilm development. Among the noble metal nanoparticles, AgNPs present a series of features like simple synthesis routes, adequate and tunable morphology, and high surface to volume ratio, intracellular delivery system, a large plasmon field area recommending them as ideal biosensors, catalysts or photo-controlled delivery systems. AgNPs are more favourable due to their characteristics such as chemically stable, reliable, cheap in production, have catalytic properties in chemical reaction, good conductivity, anti-inflammatory, and antimicrobial activity. Thus, the properties make them not only being applied in many consumers' products, food production or cosmetics but also in medical and biomedical field including in wound dressing, as antiseptics, dental fillers and as a coating materials on medical devices to prevent biofilm production by bacteria (Garvita *et al.,* 2014). Silver nanoparticles were proven having high toxicity towards many microorganisms. However, they were found to be less or non toxic towards human and other mammalian cells at low concentration (Jacob *et al*., 2013).

SYNTHESIS OF SILVER NANOPARTICLES

1) Physical methods :

Evaporation-condensation and laser ablation are the most important physical approaches. The absence of solvent contamination in the prepared thin films and the uniformity of NPs distribution are the advantages of physical synthesis methods in comparison with chemical processes.

2) 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 ($NaBH_4$), and elemental hydrogen are used for reduction of silver ions (Ag^+) in aqueous or non-aqueous solutions. These reducing agents reduce Ag^+ and lead 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 (Wiley *et.al.* ,2005).

3) Bio-based methods

A number of reports prevailed in the literatures indicate that synthesis of nanoparticles by chemical approaches are eco-unfriendly and expensive. Thus, there is a growing need to develop environmentally and economically friendly processes, which do not use toxic chemicals in the synthesis protocols. This has conducted researchers to look at the organisms. The potential of organisms in nanoparticle synthesis ranges from simple prokaryotic bacterial cells to eukaryotic fungi and plants (Mohanpuria *et.al*., 2008). Some examples of nanoparticle production include using bacteria for gold, silver, cadmium, zinc, magnetite, and iron NPs; yeasts for silver, lead and cadmium NPs; fungi for gold, silver and cadmium NPs; algae for silver and gold NPs; plants for silver, gold, palladium, zinc oxide, platinum, and magnetite NPs. Bio-based protocols could be used for synthesis of highly stable and well-characterized NPs .

Nowadays, green method of production of silver nanoparticles gains more attention over the early method. Green method includes the use of bacteria, fungi and plant extracts as the reducing agent for silver ions. In general, green method are more preferable because of they are safer to the environment, need less cost of production, not require specific condition and can produce in large quantity (Garvita *et al.,* 2014).

- **Bacteria:**

Bacteria have been explored in the synthesis of silver NPs. It was reported that highly stable silver NPs (40 nm) could be synthesized by bioreduction of aqueous silver ions with a culture supernatant of nonpathogenic bacterium, *Bacillus licheniformis* (Kalishwaralal *et.al.,* 2008). Moreover, well-dispersed silver nanocrystals (50 nm) were synthesized using the bacterium *B. licheniformis*.

- **Fungi:**

Silver NPs (5-50 nm) could be synthesized extracellularly using *Fusarium oxysporum*, with no evidence of flocculation of the particles even a month after the reaction. The long-term stability of the nanoparticle solution might be due to the stabilization of the silver particles by proteins. The morphology of NPs was highly variable, with generally spherical and occasionally triangular shapes observed in the micrographs.

Stable silver NPs could be achieved by using *Aspergillus flavus* These NPs were found to be stable in water for more than 3 months with no significant aggregation because of surface binding of stabilizing materials secreted by the fungus.Extracellular biosynthesis of silver NPs (5-25 nm) using *Aspergillus fumigatus* has also been investigated (Bhainsa *et.al.,* 2006).

- **Plant:**

Synthesis of NPs using plants is very cost effective, and thus can be used as an economic and valuable alternative for the large-scale production of NPs. *Camellia sinensis* (green tea) extract has been used as a reducing and stabilizing agent for the biosynthesis of silver NPs (Vilchis-Nestor *et.al.,* 2008). AgNP synthesis from plant extracts has been developed because it is easily obtained and safe and has large variety of metabolites supporting silver ion reduction. A mechanism proposed that phytochemicals are directly involved in the alleviation of ions and realization of AgNPs.

However, among all the green methods, plant aqueous extract are the one that is most preferable because it not require any cell culture method that can have the risk of contamination occurring. Moreover, the waste product of microorganisms needs to be properly managed because it may harmful to the environment depending on the types of microorganisms used. The production using plant aqueous extract also can be very simple and fast because plants contains high reduction potential compared to other two methods. The alkaloids and phytochemicals that present in the plant extract also can give the effect of stability towards the nanoparticles that were produced (Rajan *et al*., 2015).

Catharanthus roseus (Madagascar periwinkle or Sadabahar), which belongs to the plant family Apocynaceae, has been widely introduced as one of the important medicinal plants that is used to treat different diseases in folk medicine. The leaves and roots of *C*. *roseus* are rich in phytochemicals; for instance alkaloids, which have been demonstrated to have anticancer and antihypertensive effects (Ei-Sayed and Verpoorte 2005). The plant has a long history of use in Ayurvedic medicine, traditional Chinese medicine and other healing systems. Western medical science began researching *Catharanthus roseus* and its extracts during the 20th century, finding several compounds useful in cancer treatment. All parts of the plant have been used in regional herbal medicine, including the dried root, leaves, flowers and stalks. Alkaloids used in modern medicine are extracted from the whole dried plant. There are two common cultivars of *C. roseus* named on the basis of their flower colour, one producing pink flower “Rosea” and the other, white flowers “Alba”

Domain: Eukarya
Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Gentianales
Family: Apocynaceae
Genus: *Catharanthus*
Species: *Catharanthus roseus*

Catharanthus roseus produces more than 120 terpenoids indole alkaloids (TIAs) including vinblastine, vincristine, ajmalicine, catharantine, vincoline, vincubine, vinorelbine, vindesine and serpentine. Those alkaloids that accumulated in different parts of the plant have important pharmacological value such as antitumor, sedative and antihypertensive The most valuable alkaloids of C. roseus are the vinblastine and vincristine as they have the anticancer properties. These alkaloids are accumulated in the leaves or aerial parts of the plant and being produced in low amount by the plants (Rischer *et al*., 2006). . They are the first natural drugs that were used in the treatment of cancer and commonly been used together with the chemotherapy drugs in cancer therapy. Vinblastine was approved as a part of treatment of testicular carcinoma, Hodgkin and non-Hodgkin lymphoma, breast cancer and tumour of germ cell. Vincristine was used as treatment for acute lymphoblastic leukemia, Wilm's tumor in children, neuroblastoma, rhabdomyosarcoma, Hodgkin lymphoma and other lymphomas.

The mechanism of actions of these alkaloids is by interacting with tubulin and disrupting the mitotic spindle apparatus of the microtubules. This interaction cause blocking in mitosis process and lead the cancer cell to metaphase arrest. Since these alkaloids interact and disrupt the microtubules, they can be highly toxic not only to cancer cell but also to non-malignant cell as microtubules involve in many cell functions. Therefore, many side effects being reported that consist of vomiting, toxicity of nervous system and white blood cells, suppression of the activity of bone marrow, dyspenia, kidney problems, damage of liver cell, mouth ulcer, constipation, nausea, fever, urinary retention, abdominal cramps, and hair loss or alopecia. Hence, the usage of these alkaloids should be under controlled and correct dosage must be prescribed in order to minimize the side effects (Moudi *et al*., 2013, Nejat *et al*., 2015).

The physicochemical properties of nanoparticles are important for their behavior, bio-distribution, safety, and efficacy. Therefore, characterization of AgNPs is important in order to evaluate the functional aspects of the synthesized particles.

The present study aimed to determine the biological synthesis of AgNPs produced from *C. roseus* leaf aqueous extract and characterize these nanoparticles using UV-Vis spectroscopy, DLS and zeta potential. Moreover, it also aimed to determine antioxidant effect, and anticancer activity of synthesized AgNPs.

Biological Application Of Silver Nanoparticles:

Due to their unique properties, AgNPs have been used extensively in house-hold utensils, the health care industry, and in food storage, environmental, and biomedical applications. Herein, we are interested in emphasizing the applications of AgNPs in various biological and biomedical applications, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, and anti-angiogenic. A schematic diagram representing various applications of AgNPs.

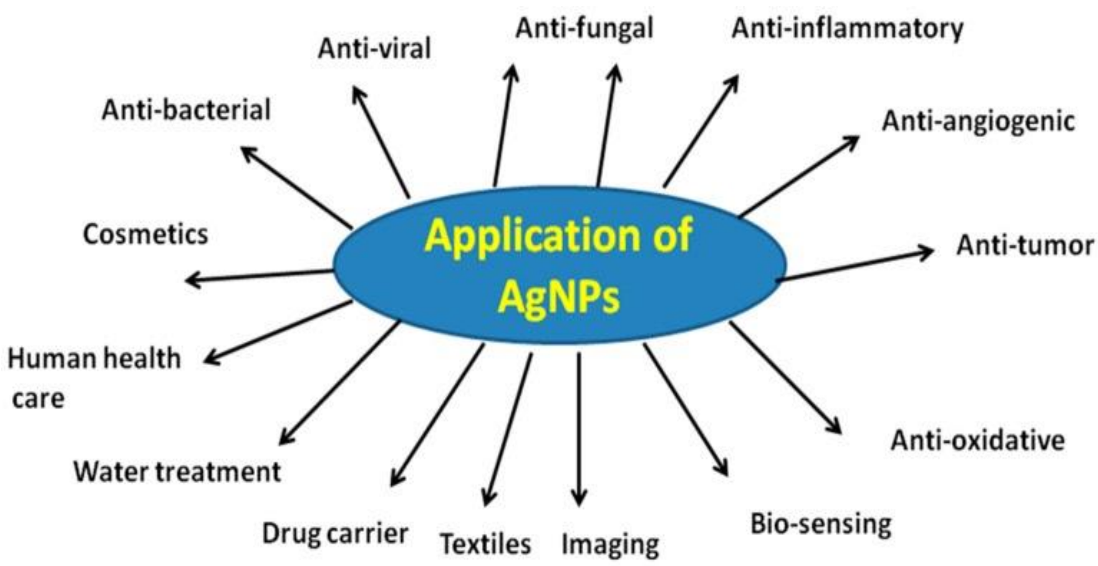


Fig.4: Various application of silver NPs

- **Scientific Applications:** Due to the surface Plasmon resonance (SPR) and surface enhanced raman scattering (SERS) properties of silver nanoparticles, they have many applications such as sensing applications including detection of DNA sequences. laser desorption/ionization mass spectrometry of peptides , colorimetric sensors for Histidine , enhanced IR absorption spectroscopy, biolabeling and optical imaging of cancer. biosensors for detection of herbicides and glucose sensors for medical diagnostics.

- **Medical Applications:** Nanosilver is used for coating such as incorporated in wound dressings, diabetic socks, scaffolds, sterilization materials in hospitals, medical textiles etc. One website claims that "the number of people using colloidal silver as a dietary supplement on a daily basis is measured in the millions".

- **Industrial Applications:**

➢ **Catalysis:** The silver nanomaterials and silver nanocomposites are used as catalyst in many such as CO oxidation, benzene oxidation to phenol, photodegradation of gaseous acetaldehyde and the reduction of the p-nitrophenol to p-aminophenol. To catalyze the reduction of dyes by sodium borohydride (NaBH4), silver nanoparticles immobilized on silica spheres etc.

➢ **Electronics:** Nanosilver has high electrical and thermal conductivity along with the enhanced optical properties leads to various applications in electronics. In nanoelectronics, silver nanowires are used as nanoconnectors and nanoelectrodes. Other applications include), optoelectronics, nanoelectronics (such as single-electron transistors and electrical connectors), data storage devices, the preparation of active waveguides in optical devices high density recording devices, intercalation materials for batteries, making microinterconnects in integrated circuits (IC) and integral capacitors etc.

- **Antibacterial Activity of AgNPs:** AgNPs seem to be alternative antibacterial agents to antibiotics and have the ability to overcome the bacterial resistance against antibiotics. Therefore, it is necessary to develop AgNPs as antibacterial agents. Among the several promising nanomaterials, AgNPs seem to be potential antibacterial agents due to their large surface-to-volume ratios and crystallographic surface structure.

- **Antifungal Activity of AgNPs:** Fungal infections are more frequent in patients who are immunosuppressed, and overcoming fungi-mediated diseases is a tedious process, because currently there is a limited number of available antifungal drugs. Therefore, there is an inevitable and urgent need to develop antifungal agents, which should be biocompatible, non-toxic, and environmentally friendly. At this juncture, AgNPs play an important role as anti-fungal agents against various diseases caused by fungi. Nano-Ag showed potent anti-fungal activity against clinical isolates and ATCC strains of *Trichophyton mentagrophytes* and *Candida species*.

- **Antiviral Activity of AgNPs:** Viral mediated diseases are common and becoming more prominent in the world; therefore, developing anti-viral agents is essential. The mechanisms of the antiviral activity of AgNPs are an important aspect in antiviral therapy. AgNPs have unique interactions with bacteria and viruses based on certain size ranges and shapes (Lok *et.al*., 2006).

- **Anti-Inflammatory Activity of AgNPs:** Inflammation is an early immunological response against foreign particles by tissue, which is supported by the enhanced production of pro-inflammatory cytokines, the activation of the immune system, and the release of prostaglandins and chemotactic substances. In order to overcome inflammatory action, we need to find effective anti-inflammatory agents. Among several anti-inflammatory agents, AgNPs have recently played an important role in anti-inflammatory field. AgNPs have been known to be antimicrobial, but the anti-inflammatory responses of AgNPs are still limited.

- **Anticancer Activity of AgNPs:** In our lifetime, 1 in 3 people has the possibility to develop cancer. Although many chemotherapeutic agents are currently being used on different types of cancers, the side effects are enormous, and particularly, administrations of chemotherapeutic agents by intravenous infusion are a tedious process Therefore, it is indispensable to develop technologies to avoid systemic side effects. At this juncture, many researchers are interested in developing nanomaterials as an alternative tool to create formulations that can target tumor cells specifically. Biologically synthesized AgNPs capable of altering cell morphology of cancer cells which is an early indicator for apoptosis.

- **Diagnostic, Biosensor, and Gene Therapy Applications of AgNPs:** The advancement in medical technologies is increasing. There is much interest in using nanoparticles to improve or replace today's therapies. Nanoparticles have advantages over today's therapies, because they can be engineered to have certain properties or to behave in a certain way. The ability of AgNPs in cellular imaging in vivo could be very useful for studying inflammation, tumors, immune response, and the effects of stem cell therapy, in which contrast agents were conjugated or encapsulated to nanoparticles through surface modification and bioconjugation of the nanoparticles. Silver plays an important role in imaging systems due its stronger and sharper plasmon resonance. AgNPs, due to their smaller size, are mainly used in diagnostics, therapy, as well as combined therapy and diagnostic approaches by increasing the acoustic reflectivity, ultimately leading to an increase in brightness and the creation of a clearer image. Nanosilver has been intensively used in several applications, including diagnosis and treatment of cancer and as drug carriers. Nanosilver was used in combination with vanadium oxide in battery cell components to improve the battery performance in next-generation active implantable medical devices. Recently, silver has been used to develop silver-based biosensors for the clinical detection of serum p53 in head and neck squamous cell carcinoma. In addition, it has been explored for the location of cancer cells, and can absorb light and selectively destroy targeted cancer cells through photothermal therapy (Loo *et.al.,* 2005).

<u>Cancer</u>

Cancer is among the most prevalent disease and one of the main killers worldwide. According to the World Health Organization (WHO), 14.1 million of new cancer cases were estimated being reported to occur worldwide and the number were expected to increase by 2030. From 100,000 populations, 205.4 cancer occurrences were reported in men and 165.3 in women in 2012. Among all the cancer death reported, lung, breast, bowel and prostate cancer were recorded as the most common causes in worldwide (International Agency for Research on Cancer, 2012, World Health Organization, 2014, Torre *et al.,* 2015).
Leukaemia was the seventh most prevalent cancer among adult age and the most common happened in children.

Cancer can begin almost anywhere in the human body, which is made from trillions of cells. Normally, human cells develop and multiply (through a technique referred to as cell department) to form new cells because the body needs them. When cells develop vintage or come to be broken, they die, and new cells take their location. Sometimes this orderly manner breaks down, and bizarre or broken cells grow and multiply once they shouldn't. These cells may additionally shape tumors that are lumps of tissue. Tumors may be cancerous or not cancerous (benign). Cancerous tumors spread into, or invade, nearby tissues and may tour to distant places within the frame to shape new tumors (a process known as metastasis). Cancerous tumors can also be known as malignant tumors. Many cancers shape solid tumors, but cancers of the blood, such as leukemia's, generally do not. Benign tumors do now not unfold into, or invade, close by tissues. When eliminated, benign tumors generally don't develop lower back, whereas cancerous tumors sometimes do. Benign tumors can on occasion be quite huge, but. Some can purpose critical symptoms or be life threatening, which includes benign tumors inside the mind.

The anticancer alkaloids Vinblastine and Vincristine are derived from leaf of Catharanthus roseus. Different percentage of the ethanolic crude extracts of Catharanthus was found to show the significant anticancer activity against numerous cell types in the in vitro condition and especially greatest activity was found against the multidrug resistant tumor types. They are also used for treatment of leukemias, lymphomas, and testicular cancer. It has been demonstrated that ethanol extracts of aerial and roots of C. roseus reduced the proliferation of the human ductal breast epithelial tumour cell lines (T47D) with a mean IC50 of 2.8% by apoptosis. Also, aqueous extract of leaves induced cell death of human leukemic T-cells (Jurkat) in a time – and dose – dependent manner with mean IC50 value of 2.38 µg/ml by DNA fragmentation. Preliminary cytotoxicity study has demonstrated further a dose independnet activity of ethanol extracts of C. roseus against HCT-116 colorectal carcinoma cell lines at 200 µg/ml.

Types of cancer:-Types of cancers are typically named for the organs or tissues in which the cancers form. For example, lung cancers starts within the lung, and brain cancers starts inside the brain. Cancers additionally can be described with the aid of the kind of cells that fashioned them, which include an epithelial cell or a squamous cell.

Here are a few categories of cancers that start in particular varieties of cells (Dubchak*, et.al.,* 2010).

- **Carcinoma: -** Cancer that starts inside the skin or in tissues that line or cowl internal organs “pores and skin, lung, colon, pancreatic, ovarian cancers," epithelial, squamous and basal cellular carcinomas, melanomas, papillomas, and adenomas.
- **Sarcoma: -** Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or different connective or supportive tissue -- "bone, soft tissue cancers," osteosarcoma, synovial sarcoma, liposarcoma, angiosarcoma, rhabdosarcoma, and fibrosarcoma.
- **Leukemia :-** Cancer that starts off evolved in blood-forming tissue along with the bone marrow and reasons massive numbers of extraordinary blood cells to be produced and input the blood -- "leukemia," lymphoblastic leukemia’s , myelogenous leukemia’s , T-cell leukemia, and bushy-mobile leukemia.
- **Lymphoma and myeloma: -** Cancers that start within the cells of the immune gadget -- "lymphoma," T-cellular lymphomas, B-mobile lymphomas, Hodgkin lymphomas, non-Hodgkin lymphoma, and lymphoproliferative lymphomas.
- **Central Nervous System Cancer: -** Cancers that start in the tissues of the brain and spinal twine - "brain and spinal cord tumors," gliomas, meningiomas, pituitary adenomas, vestibular schwannomas, number one CNS lymphomas, and primitive neuroectodermal tumors.
- **Melanoma: -**Melanoma is a type of cancer that begins in cells that end up melanocytes, which are specialized cells that make melanin (the pigment that offers skin its coloration). Most melanomas form at the pores and skin, but melanomas can also shape in other pigmented tissues, including the attention.

The phytochemicals being released in the body might not have a good efficacy towards the targeting of the cancer cells due to its low diffusion characteristics and failure of overcoming the anatomical barriers. Since the structure and nanosize of the silver particle makes it successful in easy diffusion and overcoming the barriers , it can be assumed that the green synthesis silver of nanoparticle from the catharanthus roseus leaf can target the cancer cells and have successful anticancer property.

Cancer is one of the deadly diseases, which resulted in the death of millions of people worldwide. Cervical cancer generally arises from the cervix and is caused by Human Papilloma Virus. Cervical cancer is the fourth common cause of cancer and eventually death in women. This cancer results in the formation of warts around the genital areas. However it is preventable and curable if the proper treatment is given and detected at an early stage. In this study the green synthesized nanoparticles were tested on cell cytotoxicity and apoptosis (inducing nuclear fragmentation) on HeLa cell lines. The unique morphological characteristics of silver nanoparticles may overcome barrier and reach the target cells.

Cancer Treatment

Conventional treatments that were used to treat or fight cancer include surgery, chemotherapies and radiotherapies, immunotherapy, targeted therapy, transplantation, cryosurgery, hyperthermia and photodynamic therapy.However, these conventional therapies were known having many side effects. For example, chemotherapies were not only kills the cancerous cell but also kill healthy dividing cells. This is due to its non-specific action, high toxicity effects and ability to enhance the development of resistance of the cancerous cells toward the drugs after long-term treatment. Other adverse effects of chemotherapies include thrombocytopenia, neutropenia and anaemia that can cause bleeding, infections, fluid retention, diarrhoea and urinary, kidney and bladder changes in worst case patients (International Agency for Research on Cancer, 2012, World Health Organization, 2014, Torre *et al*., 2015).

Radiotherapy has the capacity to cause shrinkage and damage of the tumour in a short period of time and that makes it as another common conventional treatment for cancer. However, like chemotherapy, the action of radiotherapy is not specific and can cause toxicity effects to the patients. Despite killing the cancerous tissues, it also can cause inhibition in healthy cells and induce resistance and mutation upon long-term exposure. In addition, the ionizing radiation of the radiotherapy can also induce damages or changes in oral mucosa, skin and salivary glands in normal tissues of nearby area. Furthermore, older patients and children usually have undesirable significant side effects and life threatening effects after treatment .

Another therapy that is used in cancer treatment is immunotherapy which used monoclonal antibody (mAb) or cytokine to specifically target the cancerous cells or boosting the immune system to fight against tumour. However, immunotherapy produces different response in different patients and the treatment also takes longer time that is about 3 to 5 years thus makes it expensive. The side effect of immunotherapy is it can induce severe, life threatening, anaphylactic reactions Immunotherapy was commonly used in late stage cancer treatment such as after radiotherapy, surgery or chemotherapy where the immune system of the patients is weakened due to the earlier conventional treatment (Jahrsdörfer and Weiner, 2008).

Due to the limitations of conventional treatment, many studies have been done involving nanotechnology and nanomaterials especially in nanoparticles to overcome the problems. One of the examples was the use of nano sized particles that has been widely used in drug delivery system for chemotherapeutic agents against cancer cells.

Nanoparticles or nano treatment is emerging as a new approach in cancer treatment because of their unique physical and chemical characteristics. They are very small in size thus makes them have large surface area to volume ratio. This property enables nanoparticles to pass through the cellular barrier easily and also able to efficiently carry conjugated compounds such as drugs, nucleic acids, antibody, proteins and probes. Moreover, the nano treatment also more favoured because of their characteristics such as longer shelf life, more stable, specific and only needed in low dosage frequency compared to conventional treatment.
Nanomaterials were already being used widely in medical practice such as in invasive imaging techniques, targeted drug delivery systems and in the development of engineered organs. An example, nanoscaled drug delivery 11 systems such as liposomes and drug-conjugated nanoparticles were used for cancer treatment in drug chemotherapy (Barkalina *et al*., 2014).

Recently, photo-based nanomedicine has gained much importance for cancer treatment among several approaches. Khlebtsov et al. developed multifunctional NPs which significantly induced cell death in HeLa cervical cancer cells. Wang et al. developed folic acid (FA)-coated AgNPs with an average size of 23 ± 2 nm showing excellent receptor-mediated cellular uptake; with this compound (FA-AgNPs), they conjugated the chemotherapeutic drug doxorubicin (DOX) by electrostatic bonding. DOX was released efficiently, and cell death was observed after 8 h. They concluded that AgNPs can be used as nanocarriers for desired drugs for cancer treatment.

Recently, nanomaterials have been used for diagnosis, treatment, and prevention of cancer using photo-based therapeutic approaches. The nanostructures are more capable of destroying the cancer cells than non-cancer cells at low irradiation power density. Recently, Mukherjee et al. used AgNPs as cancer theranostic agents; they prepared AgNPs from *Olax scandens* leaf extract and prepared AgNPs showing anticancer activity against various types of cancer cells, including A549, B16, and MCF7.

AgNPs are not only used as drug delivery devices but they also serve as drugs; therefore, they are used for therapeutics. AgNPs are well known antibacterial agents, and they also enhance the tumor-killing effects of anticancer drugs. The combination of chemotherapeutic agents with nanoparticles is a developing effective approach for the eradication of cancer, in which they are using lower doses of drugs to reduce cytotoxic effects and increase the efficacy. For example, the combination of Platinol (cisplatin) and Navelbine (vinorelbine) showed better efficiency in non-small cell lung cancer. Similarly, the combination of salinomycin (Sal) with AgNPs derived from *Typha angustifolia* plant extracts showed a synergistic cytotoxic effect in human ovarian cancer cells.

Challenges/Limitation for Cancer Therapy Using AgNPs

Nanomedicine is as one of the fast developing and promising strategies to combat cancer using metallic nanoparticles. Current treatment for cancer, such as chemo- and radiation therapy, has limitations due to unexpected drug-associated side effects, lack of specificity of low drug concentrations at the tumor target site, and the development of chemoresistance. Nanoparticle-mediated therapy is the best, most suitable, and alternative therapeutic strategy in cancer therapy. Nanoparticles (NPs) have the ability to target through passive or active targeting of particular diseased cells or tumor tissues by the encapsulation of therapeutic agents with nanoparticles, and they have been used as drug delivery systems. Although many nanoparticle-mediated strategies have been developed, heterogeneity of the tumor and its stroma is a significant challenge for nanotechnologists and clinicians to come up with specific formulations to precisely target specific cancer cells. To achieve higher specificity, reduction in toxicity, biocompatibility, safety, better efficacy, and to overcome the limitations of conventional chemotherapy, using new nanoparticles in single platform-based strategies is another challenge in cancer therapy. However, there is a need to address the challenges and limitations of using nanoparticles for cancer therapy; these include physiological barriers, limited carrying capacity, enhanced permeability and retention effect (EPR), variability of nanoparticles, and regulatory and manufacturing issues (Wicki *et.al*., 2015).

OBJECTIVE:

- To biosynthesize silver nanoparticles by using *Catharanthus roseus* leaf extract.

- To characterize silver nanoparticles by using UV-Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential.

- Evaluation of antioxidant potential of *Catharanthus roseus* leaf extract.

- To check the potential of silver nanoparticles against cervical cancer HeLa cells.

Material and Method:

- **Collection and Extraction of Catharanthus roseus leaves:**
  Fresh leaves of Catharanthus roseus was collected from the herbarium garden of Integral University Lucknow, India in Feb 2022. The young green *Catharanthus roseus* leaves have been trimmed and cleaned with tap water. Washed plant material has been dried in shadow for 10 days at room temperature and produces a fine powder with an electric grinder. The soxhlet was equipped with 25 g of powdered plant material and added 200 mL ethanol as a solvent and the soxhlet device running constantly for 48 h. The extracted material was filtered and poured in petri plate to dry then the dried samples were collected in cell culture disc.

- **Catharanthus roseus 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 analyzed on a bio spectrum-Kinetic Spectrophotometer using a quartz cuvette having the path length of 1 cm to a affirm the synthesis of *Catharanthus roseus* encapsulated silver 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 silver nanoparticles:**
  The transformation of silver salt into silver nanoparticles was investigated by using the Shimadzu UV-1601 dual-beam spectrometer. This measurement has a spatial resolution of one nanometer (200-800nm). The technique is done on the basis of reducing metal salts to synthesise AgNPs results in colour change.

- **UV-visible spectral analysis:** The UV-Visible spectral analysis for silver nanoparticle was performed. The spectral analysis was tested using UV3600—

Shimadzu UV-vis-NIR Spectrophotometer. Briefly 3ml of the reaction mixture was taken in the quartz cuvette for spectral analysis. The spectroscopic readings were taken at different time intervals of incubation (1, 2, 3, 4, 5 and 6h). Spectral analysis was done in the range of wavelengths from 300-700nm.

- **Zeta potential analysis:** To study the particle size based on the refractive index and to check the stability of the silver nanoparticles that has been synthesized from the aqueous leaf extract of Catharanthus roseus The reaction mixture was collected in 15ml centrifuge tube and centrifuged at 10,000 rpm for 20 min. Washing step was repeated for 3 times with deionized water after vortexing and sonicating every time. Finally, the supernatant was discarded and the pellet was stored in 4 degree centigrade for future use. The pellet was resuspended in deionized water and subjected for zeta potential analysis.

- **Dynamic light scattering analysis:** The dynamic light scattering (DLS) technique was also employed to estimate the particle size of the synthesised AgNPs. DLS determine the size of not only nanoparticles inner inorganic core but the solvent sheath adhered to the nanoparticles core as well.

Antioxidant activity of Catharanthus roseus leaf extract:

FRAP assay: The FRAP test is a typical Electron Transfer based method used to measure the reducing power of the complex of ferric ions (Fe^{3+})- ligand to the intense blue ferrous complex (Fe^{2+}) by means of antioxidants in acid environments. The FRAP test is performed in acidic pH conditions (pH = 3.6) to keep iron solubility. Potassium ferricyanide is the most common ferric reagent used in FRAP tests. In case of Potassium Ferricyanide, Prussian blue is obtained as a final product that shows the reducing power of the antioxidants tested.

Procedure:

- Take extract of different concentration in different test tubes (1 ml make-up).
- Add 2.5 ml PBS in all test tubes.
- Thoroughly mix it.
- Add 2.5 ml of 1% potassium ferricyanide solution in all test tubes.
- Vortex the sample prepared.

- Samples incubated at 50 degree centigrade for 20 minutes.
- Add 2.5 ml of 10% TCA in all test tubes.
- Centrifuges at 3000 RPM for 10 minutes.
- Collect the supernatant in separate test tubes.
- Add deionised water in all test tubes.
- Add 0.5 ml of ferric chloride, bluish colour will be formed.
- Take measurement at 700 nm.

Samples having more concentration will show higher absorbance.

Anticancer activity of Catharanthus roseus leaf extract on human cervical cancer cells (HeLa cells):

➢ ***MTT cell viability assay:*** The MTT assay was used as a relative measure of cell viability. Briefly, cells were seeded at the density of 2*10^4 cells/ml. Quadruple cell sample were grown in 96-well microtiter plates. After 24h, samples were exposed to different concentration of EO or standard compounds (0.39-200 microgram per ml) in a final volume of 100 microlitre of culture medium. Cells were incubated for 24h in a humidified atmosphere of 5% CO_2 at 37 degree centigrade. At the end of incubation, each well received 10 microlitre of MTT (5mg/ml in phosphate-buffered saline(PBS)), and the plates were incubated for 2 h at 37 degree centigrade. The formazan crystals formed were solubilized in 100 microlitre DMSO after aspirating the medium. The extent of MTT reduction was measured spectrophotometrically at 595 nm using a Titertek Multiscan micro Elisa, 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.

Morphological analysis:

Phase contrast microscope was used to examine the morphological alterations in HeLa cells treated with *Catharanthus roseus* extract and silver nanoparticle. Briefly, HeLa cells ($5x10^3$) were cultured in a 96 well plate for 24 h, followed by treatment of *Catharanthus roseus* extract (100-400 µg/ml) and AgNPs for 24 hr. Thereafter, phase contrast microscope was used to examine the morphology of cervical cancer (HeLa) cells.

Results:

Catharanthus roseus Mediated synthesis of AgNPs (MD-AgNPs)

This study used *Catharanthus roseus* leaf extract as a reducing sugar and capping agent ,whereas 3Mm silver nitrate (AgNO3) served as the silver precursor. The synthesis of MD-AgNPs is considered to be induced by the aqueous extracts reducing enzymes and capping agents, such as secondary metabolites. The creation of MD-AgNPs was confirmed visually by a shift in the color of the extract from green to dark brown, indicating silver reduction.

Characterization of MD-AgNPs

Due to Surface Plasmon resonance, an unusual phenomenon is seen in noble metal nanoparticles (SPR). This imbues the nanoparticles surface with the character of powerful electromagnetic fields, resulting in scattering and absorption. Thus, the formation of MD-AgNPs was confirmed herein using UV-vis spectra (Fig.). The absorption peak was observed 456nm, which corresponds to the SPR band of the MD-AgNPs. The Phyto constitution in *Catharanthus roseus* peel extract reduced the silver salt (AgNO3) into AgNPs and encapsulated the silver nanoparticle preventing the nanoparticles from the Aggregating and providing stability to the MD-AgNPs. The change in colour from light green to dark brown indicated the successful synthesis of MD-AgNPs, and the result of SPR band Confirm that at 428 and however there was no discernible peek for Catharanthus roseus 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 MD-AgNPs had an average particle size of 67 d nm as shown in figure. Furthermore, the Zeta potential of the prepared MD-AgNPs was observed at the room temperature, to be a -26.6 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**.**

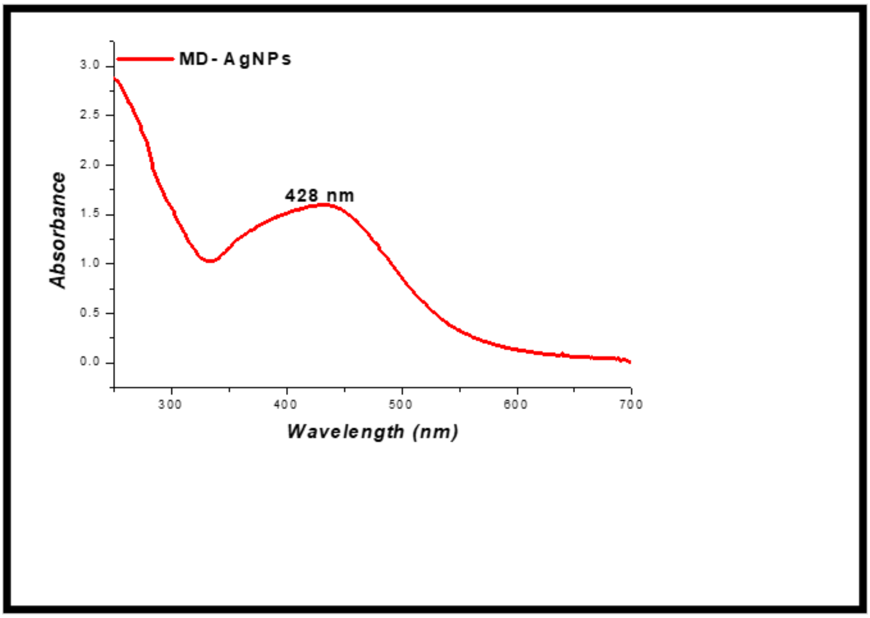


Fig 5 :- . Characterisation of MD-AgNPs under UV-Visible spectra (428 nm).

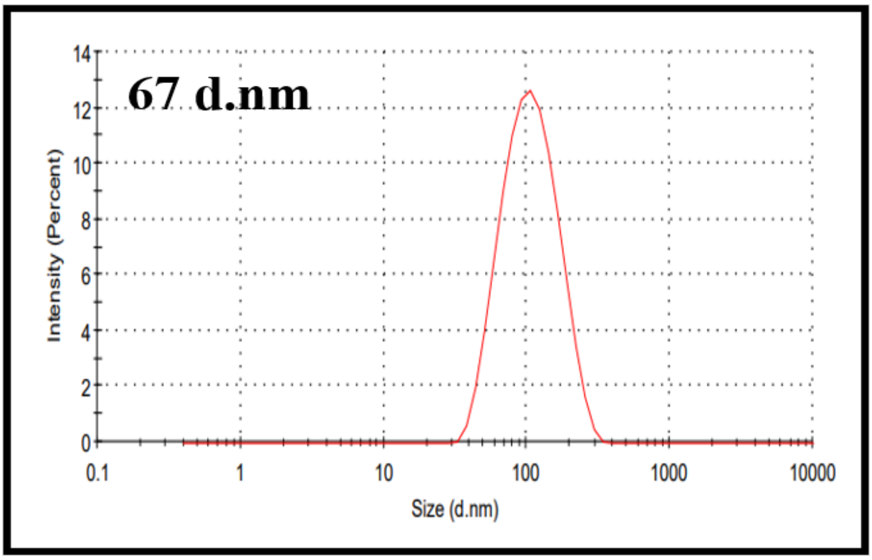


Fig 6 : DLS profile of MD-AgNPs showing size of 67 d.nm.

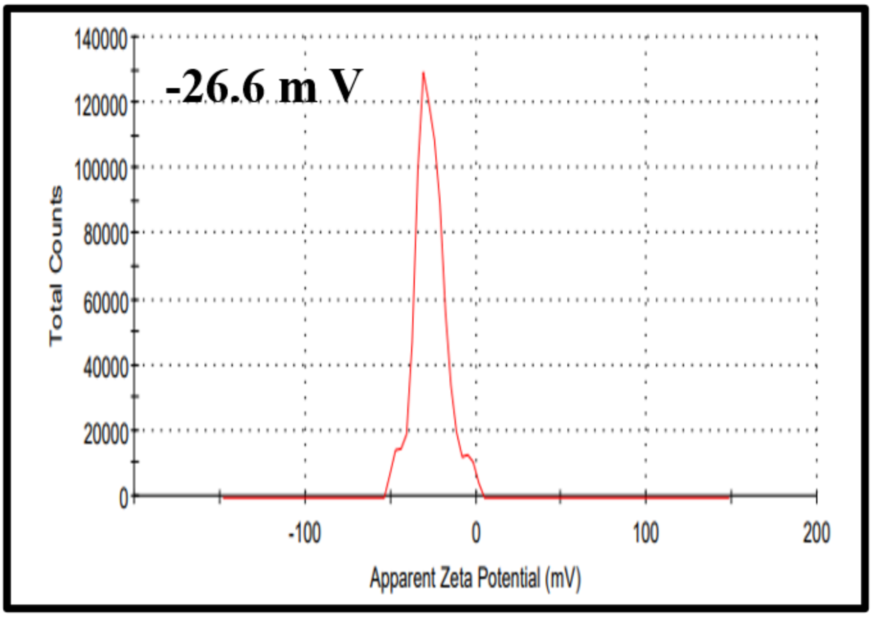
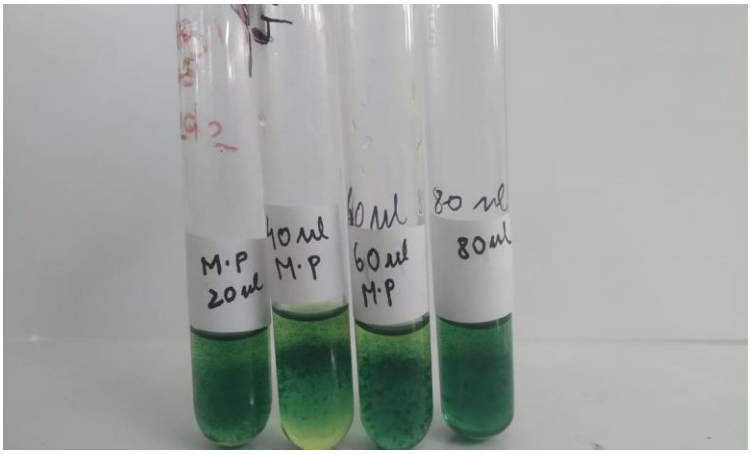


Fig 7: Zeta potential of MD-AGNPs confirmed the stability at -26.6 mV.

- **Antioxidant property**

➢ **FRAP assay:** The intensity of the blue color of sample prepared represents the concentration of the four different samples. The higher the concentration of the extract, the greater is the intensity of colour of the sample and the lesser will be its anti-oxidant properties. As the concentration increases from 20 µl towards 80 µl, the antioxidant properties decrease accordingly. The results of the absorbance (at 700 nm) of plant extract of Catharanthus roseus obtained from four different concentrations of samples (i.e. 20 µl, 40 µl, 60 µl and 80 µl) comes out to be 0.966, 1.309, 1.346 and 1.706 respectively.



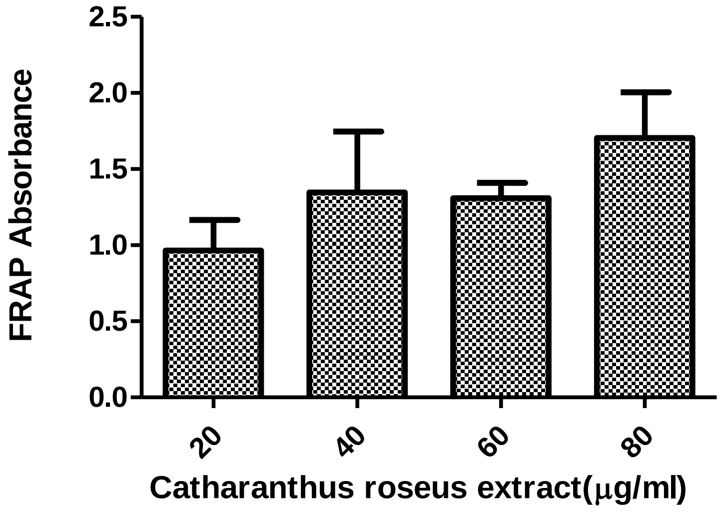
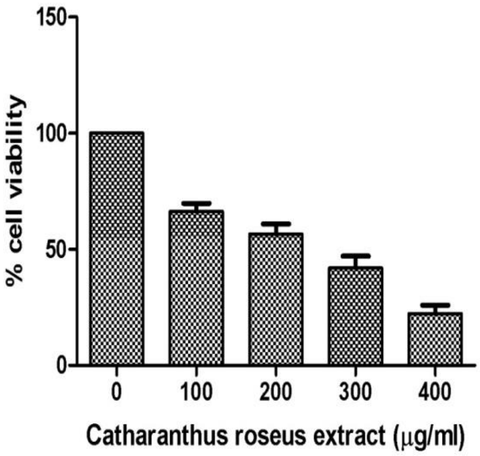
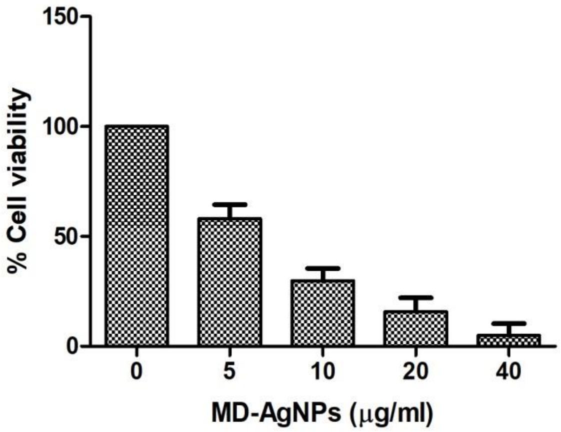


Figure 8: Antioxidant activity of *Catharanthus roseus* extract for four different concentrations

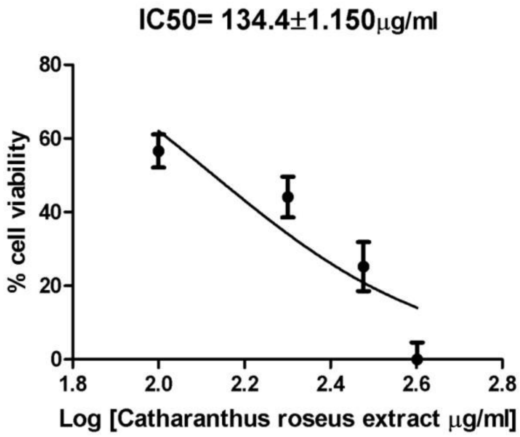
- **Anticancer property**

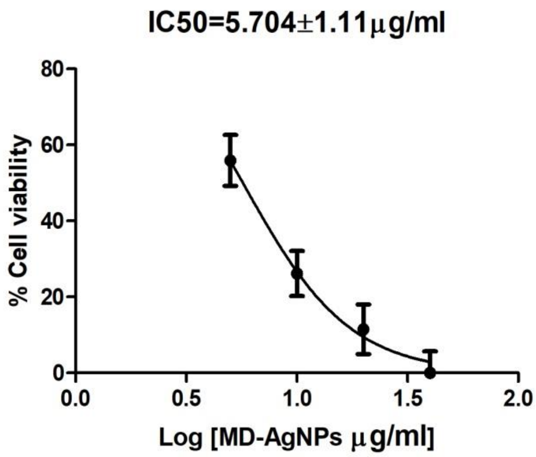
The images of the untreated and treated cervical cancer cell line (HeLa cells) showed observable morphological changes under phase contrast microscope. The untreated cells revealed progressive cell growth with intact cell morphology under microscope(figure). However, severe morphological alterations were noticed in *Catharanthus roseus* extract treated HeLa cells in a dose dependent manner (5 , 10, 20, 40). Moreover, an increase in detachment and cytoplasmic shrinkage of cells were observed in *Catharanthus roseus* extract and MD-AgNPs cervical cancer cells which resulted into greater number of floating cells. Thus, the results confirm that *Catharanthus roseus* extract and MD-AgNPs induce cytotoxicity in cervical cancer HeLa cells.





(A) (B)





(C) (D)

Fig 9: (A) Percent cell viability of HeLa cells treated with different doses of ethanolic extract of *Catharanthus roseus* (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml) and (B) MD-AgNPs (5, 20, 40 µg/ml) for 24 hr assessed by MTT assay. (C) Graph showed that *Catharanthus roseus* extract exhibited an IC50 value 134.4±1.150 µg/ml and (D) MD-AgNPs exhibit an IC50 value 5.704±1.11 µg/ml at 24 h, against HeLa cervical cancer cells. The results represented are the mean ± SEM of three independent experiments performed in triplicate.

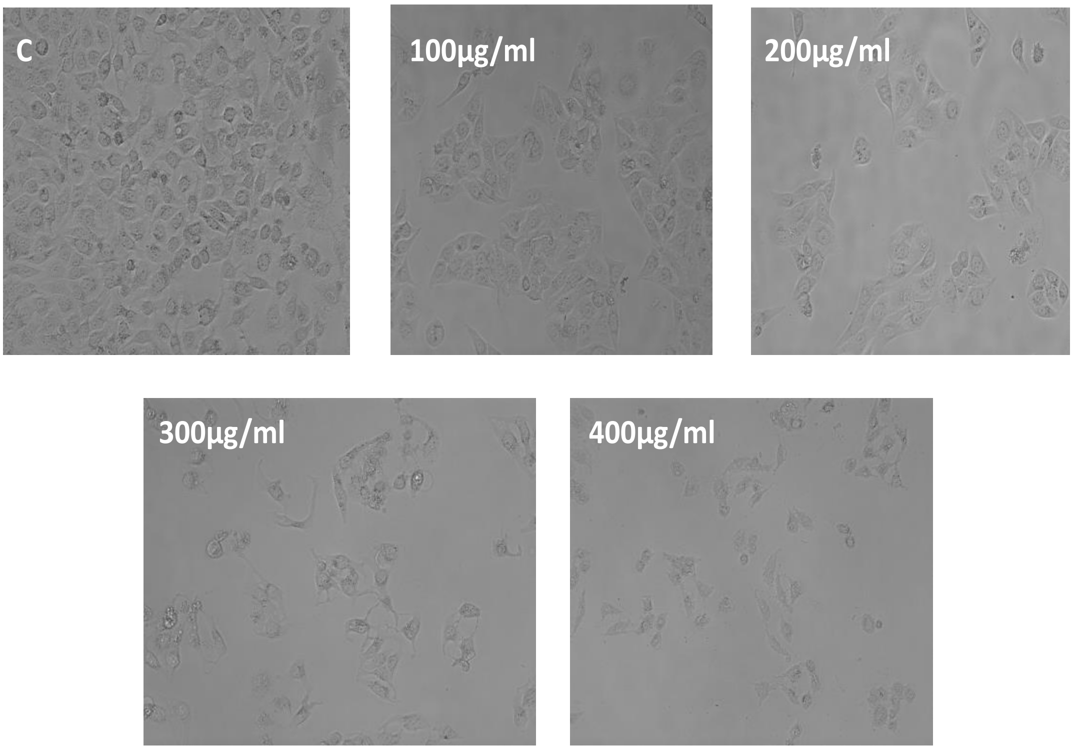


Fig.10: Phase contrast micrographs of HeLa cells treated with different doses of *Catharanthus roseus* extract (100, 200, 300, 400 µg/ml) for 24 h in a time- and dose-dependent manner. Images shown are representative of three independent experiments.

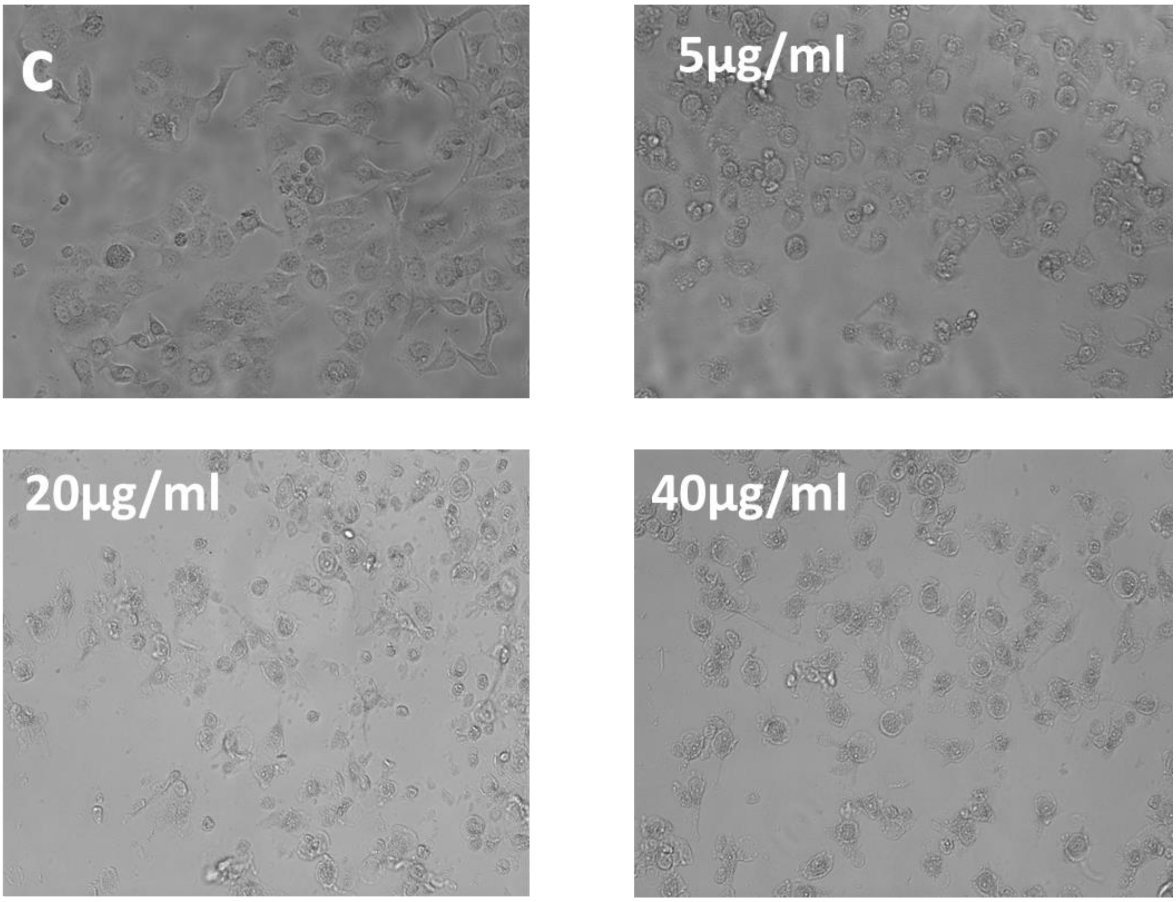


Fig.11: Phase contrast micrographs of HeLa cells treated with different doses of MD-AgNPs (5, 20, 40 µg/ml) for 24 h in a time and dose-dependent manner. Images shown are representative of three independent experiments.

Conclusion and future Perspectives:

In this study we showed Leaf extract mediated green synthesis of silver nanoparticles from *Catharanthus roseus* 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 leaf extracts of *Catharanthus roseus* plants. The Ag NPs were characterized by UV-visible spectrophotometer, particle size analyzer (DLS) and Zeta potential. This study 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. In this study we also compared the anticancer activity of *Catharanthus roseus* extract and MD-AgNPs and found that MD-AgNPs showed more appropriate anticancer activity as compare to *Catharanthus roseus* extract. 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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