A DISSERTATION ON

Murraya koenigii bark aqueous extract synthesized Silver Nanoparticles and their antibacterial activity against gram-positive and gram-negative pathogens

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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 **Mr. Siddharth Yadav**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed his four months dissertation work entitled **"*Murraya koenigii bark aqueous extract synthesized Silver Nanoparticles and their antibacterial activity against gram-positive and gram-negative pathogens"*** successfully. He has completed this work from 2 Feb to 2 June 2022 at the Department of Biosciences, Integral University, under the guidance of **Dr. Salman Khan**.

The dissertation was a compulsory part of his M.Sc. degree. I wish him good luck and a bright future.

(**Dr. Snober S. Mir**)

Head,

Department of Biosciences,

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IU/DBS/S00192/2022/CIN/221115 **June 2022**

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Mr. Siddharth Yadav**, during the months 2 Feb to 2 June 2022 reported in the present thesis was under my guidance and supervision. The results reported by him are genuine and the script of the thesis has been written by the candidate himself. The thesis entitled ***"Murraya koenigii bark aqueous extract synthesized Silver Nanoparticles and their antibacterial activity against gram-positive and gram-negative pathogens"*** is, therefore, being forwarded for acceptance in partial fulfillment of the requirements for the degree award of the student of M.Sc. Biotechnology (IV semester), Department of Biosciences, Integral University, Lucknow, (U.P).

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I would like to express my deep sense of gratitude to **Dr. Salman Khan (Assistant professor)** for their invaluable guidance throughout my dissertation work and academic session. It would have been impossible to complete this work in so short a time without his constant guidance. I wish every trainee and research student were fortunate enough to have such an affectionate guide.

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My acknowledgment will be incomplete if I do not mention my parents with whose blessing, I was able to achieve my goal successfully. There are no words to express my feelings toward them. I silently acknowledge my debt to them.

Siddharth Yadav **Date**

Introduction

Nanotechnology

The ideas and concepts behind nanoscience and nanotechnology were started by physicist Richard Feynman at an American Physical Society meeting at the California Institute of Technology on December 29, 1959, long before the term nanotechnology was used. The term nanotechnology was defined by Professor Norio Taniguchi in 1974 as “Nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or by one molecule”. It refers to research and technology development at the atomic, molecular, and macromolecular scale, leading to the controlled manipulation and study of structures and devices with length scales in the 1 to the 100-nanometer range. Objects at this scale, such as “nanoparticles,” take on novel properties and functions that differ markedly from those seen in the bulk scale. The small size, surface tailor ability, improved solubility, and multifunctionality of nanoparticles open many new research avenues for biologists. The novel properties of nanomaterials offer the ability to interact with complex biological functions in new ways operating at the very scale of biomolecules. This rapidly growing field allows cross-disciplinary researchers the opportunity to design and develop multifunctional nanoparticles that can target, diagnose, and treat diseases such as cancer. **[1]**

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale or one billionth of a meter. At these scales, consideration of individual molecules and interacting groups of molecules about the bulk macroscopic properties of the material or device becomes important, since it is control over the fundamental molecular structure that allows control over the macroscopic chemical and physical properties. Applications to medicine and physiology imply materials and devices designed to interact with the body at subcellular (i.e., molecular) scales with a high degree of specificity. This can potentially translate into targeted cellular and tissue-specific clinical applications designed to achieve maximal therapeutic effects with minimal side effects. **[2]**

Nanotechnology encompasses the fabrication and application of chemical, physical, and biological systems at scales ranging from individual molecules or atoms to submicron dimensions, and also the integration of these resulting nanomaterials into larger

systems. It has the potential to change our perspectives and expectations and provide us with the capability to resolve global issues. The discovery and use of carbon nanomaterials have allowed the introduction of many new areas of technology in Nanomedicine, biosensors, and bioelectronics. In recent years, nanotechnology has emerged as a multidisciplinary field, in which gaining a fundamental understanding of the electrical, optical, magnetic, and mechanical properties of nanostructures promise to deliver the next generation of functional materials with wide-ranging applications **[3].**

Nanoparticles

Nanoparticles are particles of sizes ranging from 1 to 100nm with one or more dimensions. The nanoparticles are generally classified into organic, inorganic, and carbon-based particles on a nanometric scale that has improved properties compared to larger sizes of respective materials. The nanoparticles show enhanced properties such as high reactivity, strength, surface area, sensitivity, stability, etc. because of their small size. The nanoparticles are synthesized by various methods for research and commercial uses that are classified into three main types namely physical, chemical and mechanical processes that have seen a vast improvement over time. **[4]**

Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industrial production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labeling and treatment of some cancers. due to their exceptional properties including antibacterial activity, high resistance to oxidation, and high thermal conductivity, nanoparticles have attracted considerable attention in recent years **[5].**

Types of nanoparticle

1-Metallic nanoparticles -These nanoparticles have been used in drug delivery,

especially in the treatment of cancer and also in biosensors. Amongst various metals, silver, gold, and selenium nanoparticles are prime for medical use. **[6]**

a-Silver- Silver nanoparticles have proved to be most effective because of their good antimicrobial efficacy against bacteria, viruses, and other eukaryotic micro-organisms **[7,8].** They are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions, etc. **[9,10].** Studies have already reported the successful biosynthesis of silver nanoparticles by plants such as Azadirachta indica **[11],** Capsicum annuum **[12],** and Carica papaya **[13]**.

b-Gold- Gold nanoparticles (AuNPs) are used in immunochemical studies for the identification of protein interactions. They are used as lab tracers in DNA fingerprinting to detect the presence of DNA in a sample. They are also used for the detection of aminoglycoside antibiotics like streptomycin, gentamycin, and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and identification of different classes of bacteria.**[14,15]**

2-Alloy- Alloy nanoparticles exhibit structural properties that are different from their bulk samples **[16].** Since Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity **[17],** Ag flakes are most widely used. Bimetallic alloy nanoparticles' properties are influenced by both metals and show more advantages over ordinary metallic NPs **[18]**.

3-Magnetic- Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (MRI) **[19].**

Platform of nanoparticles

There are diverse types of NPs platforms that differ in size, shape, compositions, and functionalities. Those NPs platforms are discussed below-

1) Liposomes-Liposomes are the first platform for NPs. In 1965, liposomes were described as a model of the cellular membrane **[20].** After that liposomes were

used for genetic and drug delivery. Liposomes are vesicles in spherical shapes which contain lipids of single or multiple bilayer structures that can assemble themselves in aqueous systems**[21].** Liposomes can be used for targeting ligands to upsurge the buildup of diagnostic and therapeutic agents with anticipated cells. Now, 12 liposome-based therapeutic drugs are clinically approved.

2) Albumin-bound- Albumin-bound NPs use the endogenous albumin trails which transport hydrophobic molecules in the bloodstream **[22].**Its quandaries with hydrophobic molecules with non-covalent reversible binding and dodging solvent-based toxicities for therapeutics. So, this platform is adapted for drug delivery **[23].**

3) Polymeric- These NPs are formed from biocompatible and biodegradable polymers which are used as a therapeutic carriers **[24].**

Polymeric NPs are verbalized through block-polymers of diverse hydrophobicity **[25].** This NPs design is useful because of the slow and controlled release of drugs at required sites.

4) Quantum dots- QDs are semiconductor particles and their size is less than 10 nm in diameter. QDs show unique size-dependent electronic and optical properties **[26].** Mostly the quantum dots consist of cadmium selenide as the core and a zinc selenide as a cap or a shell. They are used in biological research as fluorescence imaging cell labeling and biomolecule tracking **[27].**

5) Iron oxide- Iron oxide NPs are studied as passive and active targeting imaging agents because they are superparamagnetic. They have an iron oxide core with a hydrophilic coat of dextran or another biocompatible compound to increase their stability. They are mostly used in MRI **[28,29].**

Review of literature

Murraya koenigii (Curry plant)

Introduction- *Murraya koenigii* have a lot of bioactive principles due to which plant has been proven as the medicinally important plant. There are different forms of *Murraya koenigii* due to which they are found as the useful plant such as extract, essential oil, or directly used due to the presence of the following active constituents bismahanine, murrayanine, murrayafoline-A, bi-koeniquinone-A, bismurrayaquinone, mukoenine-A, mukoenine-B, mukoenine-C, murrastifoline, Murrayazolinol, murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline, xynthyletin, koenigine. For therapeutic or prophylactic purposes medicinal plants are used. For the therapeutic properties of medicinal plants presence of secondary metabolites plays a very important role such as alkaloids, flavonoids, terpenoids, vitamins, tannins, etc., these all are the secondary metabolites of the plant as an active constituents **[30].** All these secondary metabolites can act as reducing, capping, and stabilizing agents.**[31]**

Fig. 1. *Murraya koenigii* tree

Biological source- The species' name commemorates the botanist Johann König. The genus Murray commemorates Swedish physician and botanist Johann Andreas Murray who died in 1791. Hence the botanical name of the curry leaves is *Murraya koenigii*. **[32]**

Taxonomic status-

a. Kingdom – Plantae

b. Sub-kingdom – Tracheobionta

c. Super division – Spermatophyta

d. Division – Magnoliophyta

e. Class – Magnoliopsida

f. Subclass – Rosidae

g. Order – Sapindales

h. Family – Rutaceae

i. Genus – Murraya

j. Species – koenigii

Distribution-*Murraya koenigii* originates from the east and south parts of India, Pakistan, Sri Lanka, China, and Hainan but is widely cultivated in South-East Asia and some parts of the United States and Australia. It grows throughout India up to the height of 1500 to 1655m from sea level and in the Andaman Islands. It is also available in other parts of the Asian region like in moist forests of 500-1600m height in Guangdong, Shanan, S Yunnan (Xishuangbanna), Bhutan, Laos, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam.

Plant description-

1) Tree-*Murraya koenigii* is a semi-deciduous, unarmed aromatic small spreading shrub or tree with a strong woody stem but slender with the stem which is dark green to brownish the tree is 4–8.7m (13–31 feet) tall, with a trunk up to 81cm diameter. The diameter of the main stem is about 16cm.

2) Flower-The flowers of curry leaves are small, white fragrant, and funnelshaped, regular, pentamerous, stalked, complete, ebracteate, hypogynous, persistent, inferior, green, corolla, polypetalous, androecium, polyandrous, lanceolate, stigma, bright, sticky, style, short, ovary, inflorescence, the diameter of a flower is 1.12 cm in the fully opened form, each cluster bear approximately 60 to 90 flowers at a time after flowering at once, 5-lobed calyx, with petals in having length 5 mm and the petals are 5 in number, with stamen in

number 10. Curry tree flowers have a sweet fragrance, bisexual with self-pollinated for produce black berries in small size with shiny appearance containing a large visible seed with the number.

3) Leaf- Curry leaves are aromatic having a characteristic aroma, leaves of curry leaves are shiny and smooth with paler undersides. Leaves are pinnate, exstipulate, having reticulate venation, and having ovate-lanceolate with an oblique base with 11-21 leaflets whose size description is each leaflet is 0.79–1.57inch long and 0.39–0.79 inch broad. Leaflets are short-stalked, alternate, gland-dotted, and have a 0.5-cm-long petiole. The leaf margins are irregularly serrated.

4) Stem- The stem of *Murraya koenigii* is brown to dark green, with dots on the bark like a small node on it, when the bark was peeled off longitudinally under the exposing the white wood underneath; the girth of the main stem is 16cm up to 6 meters in height and 15 to 40cm in diameter.

5) Bark-The chemical constituents of matured stem and bark of *Murraya koenigii* are carbazole alkaloids, coumarin galactoside, Carbazole carboxylic acid, glycolipids, Phospholipids, etc.

Uses of *Murraya koenigii*-

- **Anti-Diabetic-**The chemical constituents of curry plant-like Koenimbidine, Murrayacine, and Murrayazolinine have been found to an anti-diabetic chemically decreasing oxidative stress by acting on paraoxonase I activity.
- **Anti-Cancer Activity-** Mahanimbine, Girinimbine, Mahanine, and Murrayafoline have been found to possess anti-cancer activity by increasing the death of cancerous cell proteasome inhibitors.
- **Anti-Alzheimer's Activity-** Isomahanimbine, and Murrayazolidine possess the activity against Alzheimer’s disease by improving the values of protective antioxidants.
- **Neuro-Protective Activity-**The curry plant possesses neuroprotective activity by some biomolecules such as Koenimbine, Koenigicine, and Clausazoline-K by decreasing glycemic levels.
- **Nephroprotective Activity-**Koenimbidine which is a carbazole alkaloid found in the stems and leaves of the curry plant has been detected as a nephroprotective agent by having renoprotective activity against unilateral renal ischemia.
- **Anti-microbial activity-** The extract of curry plant has been found to possess antimicrobial activity against Staphylococcus aureus, Escherichia

coli, Salmonella spp, etc.

Other uses of *Murraya koenigii*-

- Essential oil *Murraya koenigii* is used as sun protection and erythema agent in the formulation.
- Curry leaf oil in your regular skin care cream or lotion helps by applying it to the affected area to cure skin problems such as pimples, athlete's foot, ringworm, itches, acne, boils, and septic wounds and burns.
- Fresh leaves, dried leaf powder, and essential oil of curry leaf are widely used as flavoring soups, curries, fish, meat dishes, eggs dishes, traditional curry powder blends, seasoning, and ready-to-use other food preparations.
- The possibility of incorporating dried curry leaf powder in common dishes increases the sources of micronutrients.
- The aqueous extract of *Murraya koenigii* shows Larvicidal, pupicidal repellent, and anti-vector activity against the larvae and pupae are seen.**[33]**

History of Nanotechnology-

The development in the field of nanotechnology started in 1958 and the various stages of development have been summarized in the table below.

Table 1: Periodical development in nanotechnology

Year	**Development in nanotechnology**
1959	R. Feynman initiated the thought process
1974	The term nanotechnology was used by Taniguchi for the first time.
1981	IBM Scanning Tunneling Microscope
1986	First book on nanotechnology Engines of Creation published by K. Eric Drexler,
	Atomic Force Microscope
1989	IBM logo was made with individual atoms
1991	S. Iijima discovered the Carbon Nanotube for the first time
1999	1st nanomedicine book by R. Freitas "Nanomedicine" was published
2000	For the first time, National Nanotechnology Initiative was launched

2001	For developing the theory of nanometer-scale electronic devices and for synthesis and characterization of carbon nanotubes and nanowires, the Feynman Prize in Nanotechnology was awarded
2002	Feynman Prize in Nanotechnology was awarded for using DNA to enable the self-assembly of new structures and for advancing our ability to model molecular machine systems.
2003	Feynman Prize in Nanotechnology was awarded for modeling the molecular and electronic structures of new materials and for integrating single molecule biological motors with nano-scale silicon devices.
2004	The first policy conference on advanced nanotech was held. The first center for nanomechanical systems was established, Feynman Prize in Nanotechnology was awarded for designing stable protein structures and for constructing a novel enzyme with an altered function.
2005 - 2010	3D Nanosystems like robotics, 3D networking, and active nano products that change their state during use were prepared.
2011	The era of molecular nanotechnology started

Applications of Nanotechnology-

Nanotechnology in health and medicine- Even today various diseases like diabetes, cancer, Parkinson's disease, Alzheimer's disease, cardiovascular diseases, and multiple sclerosis as well as different kinds of serious inflammatory or infectious diseases (e.g. HIV) constitute a high number of serious and complex illnesses which are posing a major problem for the mankind. Nanomedicine is an application of nanotechnology which works in the field of health and medicine. Nano-medicine makes use of nanomaterials and nanoelectronic biosensors. In the future, nanomedicine will benefit molecular nanotechnology. The medical area of nanoscience application has many projected benefits and is potentially valuable for all human races. With the help of nanomedicine early detection and prevention, improved diagnosis, proper treatment, and follow-up of diseases are possible. Certain nano-scale particles are used as tags and labels, biological can be performed quickly, and the testing has become more sensitive and more flexible.

Gene sequencing has become more efficient with the invention of nano devices like gold nanoparticles, these gold particles when tagged with short segments of DNA can be used for the detection of genetic sequence in a sample.

Nanotechnology in energy and environment- Nanotechnology will play a critical role in the coming 50 years by protecting the environment and providing sufficient energy for a growing world. The advanced techniques of nanotechnology can help storage of energy, its conversion into other forms, eco-friendly manufacturing of materials, and better enhanced renewable energy sources. Nanotechnology can be used for less expensive energy production and renewal energies, in solar technology, nano-catalysis, fuel cells, and hydrogen technology. Carbon nanotube fuel cells are used for the storage of hydrogen, thus finding application in power cars. Nanotechnology is used on photovoltaics, for making them cheap, lightweight, and more efficient, which can reduce the combustion of engine pollutants by nanoporous filters, and can clean the exhaust mechanically, with the help of catalytic converters made up of nanoscale noble metal particles and catalytic coatings on cylinder walls and catalytic nanoparticles as an additive for fuels.

Drug delivery- In nanotechnology nanoparticles are used for site-specific drug delivery. In this technique, the required drug dose is used and side effects are lowered significantly as the active agent is deposited in the morbid region only. This highly selective approach can reduce costs and pain to the patients. Thus, a variety of nanoparticles such as dendrimers, and nanoporous materials find applications. Micelles obtained from block co-polymers are used for drug encapsulation. They transport small drug molecules to the desired location. Similarly, nanoelectromechanical systems are utilized for the active release of drugs. Iron nanoparticles or gold shells are finding important applications in cancer treatment. A targeted medicine reduces drug consumption and treatment expenses, making the treatment of patients cost-effective. **[35]**

Sports Equipment- Nanoparticles are added to materials to make them stronger whilst often being lighter. They have been used in tennis rackets, golf clubs, and shoes.

Catalysis- Catalysis is an essential use of metal NPs. Because of the large surface area of nanoparticles, it shows effective potential as a catalyst. Several investigators suggested that metal nanoparticles are very useful catalysts for the reason that a substantial number of atoms remain at the surface, so these surface atoms are available for the chemical transformation of the substrate. Different nanomaterials

are used as a catalyst including metals and their oxides, sulfides, and silicates. Catalyst activity can be defined by Turn over Number (TON) and its efficiency by Turn over Frequency (TOF).

Nanotechnology in Cosmetics- Nanotechnology, and nanomaterials are found to be useful in several cosmetics products like conditioners, make-up, suntan lotion, and hair care products. Cosmetics are applied to the stratum corneum, known as dead cells, which is used to shield the body from the in-filtration of foreign materials including cosmetics. **[36]**

Classification of NPs-

The nanoparticles are generally classified into organic, inorganic, and carbon-based.

A. **Organic Nanoparticles-** Dendrimers, micelles, liposomes, ferritin, etc. are commonly known the organic nanoparticles or polymers. These nanoparticles are biodegradable and non-toxic, and some particles such as micelles and liposomes have a hollow core, also known as nanocapsules, and are sensitive to thermal and electromagnetic radiation such as heat and light **[37]**. These unique characteristics make them an ideal choice for drug delivery. The drug-carrying capacity, stability, and delivery systems, either entrapped drug or adsorbed drug system determine their field of applications and their efficiency apart from their normal characteristics such as the size, composition, surface morphology, etc. Organic nanoparticles are most widely used in the biomedical field, for example, in drug delivery systems as they are efficient and also can be injected into specific parts of the body which is also known as targeted drug delivery.

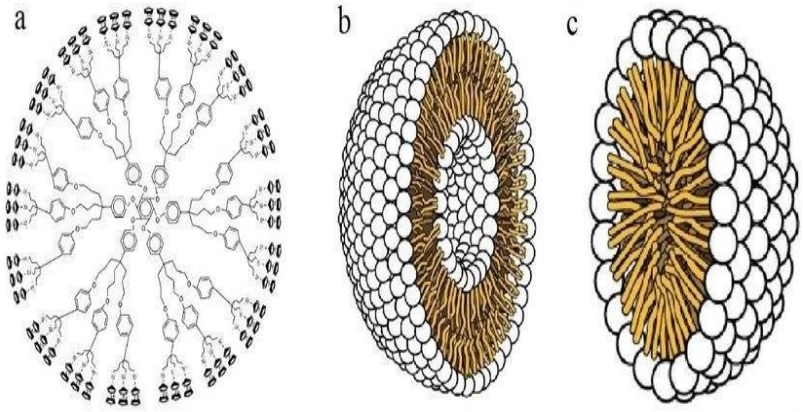

Fig. 2. Organic NPs,a-Dendrimers, b- liposomes, c- Micelles

6) **Inorganic nanoparticles-** Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide-based nanoparticles are generally categorized as inorganic nanoparticles**.**

   a. **Metal-based**- nanoparticles that are synthesized from metals to nanometric sizes either by destructive or constructive methods are metal-based. Almost all metals can be synthesized into their nanoparticles **[38]**. The commonly used metals for nanoparticle synthesis are aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn). The nanoparticles have distinctive properties such as sizes as low as 10 to 100nm, surface characteristics like the high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline, and amorphous structures, shapes like spherical and cylindrical and color, reactivity, and sensitivity to environmental factors such as air, moisture, heat, sunlight, etc.

   b. **Metal oxides based**- The metal oxide-based nanoparticles are synthesized to modify the properties of their respective metal-based nanoparticles, for example, nanoparticles of iron (Fe) instantly oxidize to iron oxide (Fe_2O_3) in the presence of oxygen at room temperature increasing its reactivity compared to iron nanoparticles. Metal oxide nanoparticles are synthesized mainly due to their increased reactivity and efficiency **[39]**. The commonly synthesized are Aluminum oxide (Al_2O_3), Cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite (Fe_3O_4), Silicon dioxide (SiO_2), and Titanium oxide (TiO_2),

Zinc oxide (ZnO).

7) **Carbon-based-** The nanoparticles made completely of carbon are known as carbon-based **[40]**. They can be classified into fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers, and carbon black and sometimes activated carbon in nano size.

- **Fullerenes-** Fullerenes (C60) is a carbon molecule that is spherical and made up of carbon atoms held together by sp^2 hybridization. About 28 to 1500 carbon atoms from the spherical structure with diameters up to 8.2 nm for a single layer and 4 to 36 nm for multi-layered fullerenes.
- **Graphene-** Graphene is an allotrope of carbon. Graphene is a hexagonal network of honeycomb lattices made up of carbon atoms on a two-dimensional planar surface. Generally, the thickness of the graphene sheet is around 1 nm.
- **Carbon Nano Tubes (CNT)-** Carbon Nano Tubes (CNT), a graphene nano foil with a honeycomb lattice of carbon atoms are 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 micrometers to several millimeters. The ends can either be hollow or closed by a half fullerene molecule.
- **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.
- **Carbon black-** An amorphous material made up of carbon, generally spherical with diameters from 20 to 70 nm. The interaction between the particles

  is so high that they are bound in aggregates and around 500 nm agglomerates are formed.**[41]**

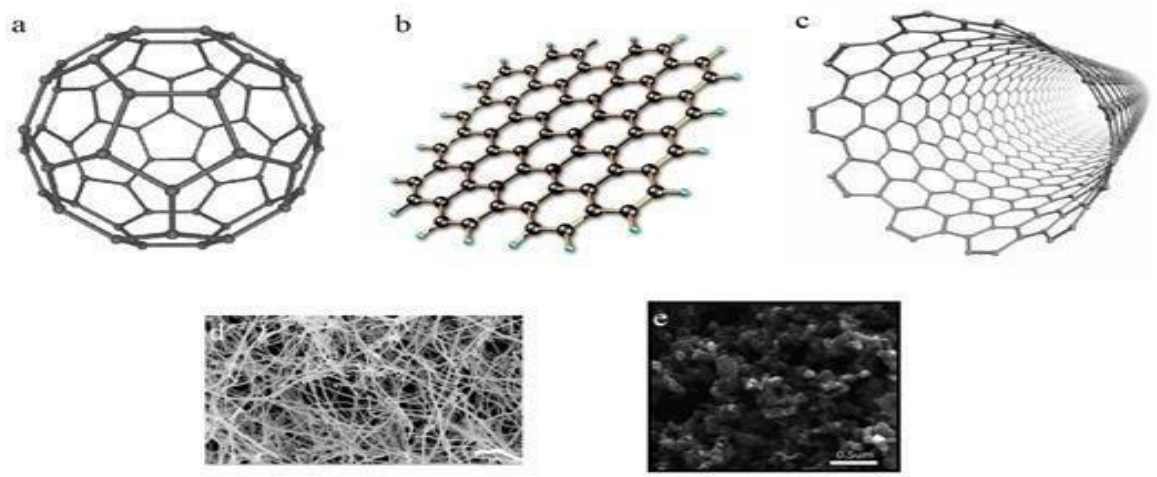

Fig. 3. Carbon-based NPs, a- fullerenes, b- graphene, c-carbon nanotubes, d-carbon nanofibers, e-carbon black

Synthesis of Nanoparticles-

NPs can be prepared by an enormous variety of methods which usually are categorized into two main synthetic routes which are top-down and bottom-up approaches.

1. **Top-down approach-** In the top-down approach, NPs are obtained from their bulk materials using different methods and techniques like thermal decomposition, irradiation, laser ablation, arc discharge, etc.
2. **Bottom-up approach-** In bottom-up (chemical and biological), NPs are obtained from the basic building blocks which react to generate NPs of the desired shape and size.
   - i) **Chemical synthesis-** Generally, the chemical synthesis of NPs employs the following three main components: (i) metal precursors, (ii) reducing agents, and
     (iii) stabilizing/capping agents. The problems associated with chemical methods are extensive use of toxic chemicals, non-eco-friendly nature of the process, need for expensive chemicals with high energy input, the requirement of sophisticated instrumentation, and further lead to the presence of some toxic chemicals adsorbed on the surface that could produce intolerable toxicity to humans and adverse effects in biomedical applications**. [42]**
   - ii) **Biological/ green synthesis-**green synthesis refers to the recruitment of biogenic matter including plant extracts, biopolymers, and microbial

sources like bacteria, fungi, algae, and yeast for nanomaterials fabrication. The development of biocompatible, non-toxic, and eco-friendly methods for the synthesis of NPs is a topic of concern in green chemistry**. [43]** The advancement of green synthesis of NPs is progressing as a key branch of nanotechnology; where the use of biological entities like micro-organisms, plant extract, or plant biomass for the production of NPs could be an alternative to chemical and physical methods in an eco-friendly manner**. [44]**

The use of medicinal plant parts like stem, root, leaf, flower, fruit, seed, bark, etc. for the synthesis of NPs is a quite novel method leading to truly green chemistry compared to other methods like chemical and physical methods. **[45]** In the biosynthesis method, plant extract has been used as reducing, capping, and stabilizing agents for the synthesis of NPs due to their reducing properties. **[46]** The main mechanism considered for the green synthesis of NP's process is plant-assisted reduction due to phytochemicals present in extracts. **[47]** Biomolecules like alkaloids, flavonoids, terpenoids, amino acids, tannins, saponins, phenols carbohydrates, etc. are present in the plant material and can act as reducing, capping, and stabilizing agents. **[48]**

Advantages of Green synthesis over Chemical synthesis-

(i) Green synthesis is simple and usually involves a one-pot reaction;

(ii) it is amenable to scale up;

(iii) the toxicity associated with hazardous chemicals is eliminated,

(iv) green biological entities can be used as reducing and capping agents, and

8) Finally, the process is cost-effective, require little intervention or input of energy, uses the renewable resource, is an environmentally friendly method and it is not necessary to use high pressure, energy, temperature, and toxic chemicals. **[49]**

Gold Nanoparticles-

Properties of gold nanoparticles are different from their bulk form because bulk gold is yellow solid and it is inert while gold nanoparticles are wine red solution and are reported to be anti-oxidant. Interparticle interactions and assembly of gold nanoparticle networks play a key role in the determination of the properties of these nanoparticles. **[50]** Gold nanoparticles exhibit various sizes ranging from 1

nm to 8 µm and they also exhibit different shapes such as spherical, sub-octahedral, octahedral, decahedral, icosahedral multiple twined, multiple twined, irregular shape, tetrahedral, Nano triangles, nano prisms, hexagonal platelets, and Nanorods.

Gold nanoparticles are widely used in biomedical science including tissue or tumor imaging, drug delivery, photothermal therapy, and immunochromatographic identification of pathogens in clinical specimens due to the surface plasmon resonance (SPR) **[51]**

Fig. 4. Gold colloids at different

Wavelengths-

Optical and electronic properties of Gold Nanoparticles-

Gold nanoparticles' interaction with light is strongly dictated by their environment, size, and physical dimensions. Oscillating electric fields of a light ray propagating near a colloidal nanoparticle interact with the free electrons causing a concerted oscillation of electron charge that is in resonance with the frequency of visible light. These resonant oscillations are known as surface plasmons. For small (~30 nm) monodisperse gold nanoparticles, the surface plasmon resonance phenomenon causes absorption of light in the blue-green portion of the spectrum (~450 nm) while red light (~700 nm) is reflected, yielding a rich red color. As particle size increases, the wavelength of surface plasmon resonance-related absorption shifts to longer, redder wavelengths. Red light is then absorbed, and blue light is reflected, yielding solutions with a pale blue or purple color (**Figure 5**). As particle size continues to increase toward the bulk limit, surface plasmon resonance wavelengths move into the IR portion of the spectrum and most visible wavelengths are reflected, giving the nanoparticles clear or translucent color. The

surface plasmon resonance can be tuned by varying the size or shape of the nanoparticles, leading to particles with tailored optical properties for different applications.

Fig. 5. Colors of various sized monodispersed gold **Nanoparticles**

Applications of Gold Nanoparticles-

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

1. **Electronics** - Gold nanoparticles are designed for use as conductors from printable inks to electronic chips. As the world of electronics becomes smaller, nanoparticles are important components in chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip.
2. **Photodynamic Therapy** - Near-IR absorbing gold nanoparticles (including gold nanoshells and nanorods) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these nanoparticles to eradicate targeted tumors. When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.
3. **Therapeutic Agent Delivery** - Therapeutic agents can also be coated onto the surface of gold nanoparticles. The large surface area-to-volume ratio of gold

(v) **Transmission Electron Microscopy (TEM)-** Transmission electron microscopy (TEM) is a high magnification measurement technique that images the transmission of a beam of electrons through a sample. Amplitude and phase variations in the transmitted beam provide imaging contrast that is a

function of the sample thickness (the amount of material that the electron beam must pass through) and the sample material (heavier atoms scatter more electrons and therefore have a smaller electron mean free path than lighter atoms). Because this technique uses electrons rather than light to illuminate the sample, TEM imaging has a significantly higher resolution than light-based imaging techniques. Successful imaging of nanoparticles using TEM depends on the contrast of the sample relative to the background. Samples are prepared for imaging by drying nanoparticles on a copper grid that is coated with a thin layer of carbon. Materials with electron densities that are significantly higher than amorphous carbon are easily imaged. These materials include most metals (e.g., silver, gold, copper, aluminum), most oxides (e.g., silica, aluminum oxide, titanium oxide), and other particles such as polymer nanoparticles, carbon nanotubes, quantum dots, and magnetic nanoparticles. TEM imaging is the preferred method to directly measure the particle size, grain size, size distribution, and morphology of nanoparticles. Sizing accuracy is typically within 3% of the actual value.

(vi) **Spectroscopic Analysis (UV-Visible Spectroscopy)-**UV/Visible spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light). In its simplest form, a sample is placed between a light source and a photodetector, and the intensity of a beam of UV/visible light is measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength-dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a buffer blank to guarantee that spectral features from the buffer are not included in the sample extinction spectrum. Gold and silver plasmonic nanoparticles have optical properties that are sensitive to size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV/Vis spectroscopy a valuable tool for identifying, characterizing, and studying nanomaterials. nanoparticles enable their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers).

1. **Sensors** - Gold nanoparticles are used in a variety of sensors. For example, a colorimetric sensor based on gold nanoparticles can identify if foods are suitable for consumption. Other methods, such as surface-enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds. This strategy could also be used for the detection of proteins, pollutants, and other molecules label-free.
2. **Probes** - Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy. The scattered colors of gold nanoparticles are currently used for biological imaging applications. Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.
3. **Diagnostics** - Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also common in lateral flow immunoassays, a common household example being the home pregnancy test.
4. **Catalysis** - Gold nanoparticles are used as catalysts in several chemical reactions. The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a reaction (nitrogen oxides). Gold nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry. **[52]**

Characterization of NPs-

(vii) Characterization of NPs is based on the size, morphology, and surface charge, using advanced microscopic techniques such as Atomic Force Microscopy, Scanning Electron Microscopy, etc. affect the physical stability and the in vivo distribution of NPs. Properties like surface morphology, size, and overall shape, are determined by electron microscopic techniques. Features such as physical stability and Re- dispersibility of the polymer dispersion as well as their in vivo performance are affected by the surface charge of NPs. Different characterization tools and methods for NPS are

(viii) **Dynamic Light Scattering (DLS)-** Dynamic Light Scattering (DLS) is an important tool for characterizing nanoparticles and other colloidal solutions.

DLS measures light scattered from a laser that passes through a colloidal solution. By analyzing the modulation of the scattered light intensity as a function of time, information can be obtained on the size of the particle in the solution. A DLS autocorrelation function. The time delay at which the function decreases correspond to the nanoparticle diffusion rate. The analysis is based on the diffusive motion of particles in solution (Brownian motion) in which larger particles will move more slowly and scatter more light than smaller particles. The hydrodynamic diameter (the diameter of a hypothetical nonporous sphere that diffuses at the same rate as the particles being characterized) can be calculated from the time dependence of the scattering intensity measurements.

(ix) **Zeta Potential**- Zeta potential (also known as the electrokinetic potential) is a measure of the “effective” electric charge on the nanoparticle surface and quantifies the charge stability of colloidal nanoparticles. When a nanoparticle has a net surface charge, the charge is “screened” by an increased concentration of ions of opposite charge near the nanoparticle surface. This layer of oppositely charged ions moves with the nanoparticle, and together the layer of surface charge and oppositely charged ions are referred to as the electrical double layer. The Zeta Potential is a measure of the difference in potential between the bulk fluid in which a particle is dispersed and the layer of fluid containing the oppositely charged ions that is associated with the nanoparticle surface. Particles with a positive Zeta Potential will bind to negatively charged surfaces, and vice versa. The magnitude of the Zeta Potential provides information about particle stability, with higher magnitude potentials exhibiting increased electrostatic repulsion and therefore increased stability. 0-5 mV: Particles tend to agglomerate or aggregate 5-20 mV: Particles are minimally stable 20-40 mV: Particles are moderately stable 40+ mV: Particles are highly stable.

Objectives

Objectives-

- Synthesis of AgNPs by *Murraya koenigii* bark aqueous extract.
- Characterization of synthesized AgNPS by UV-Vis spectroscopy, FTIR, DLS, Zeta Potential, and TEM.
- Antibacterial activity of AgNPs against the gram-positive and gram-negative pathogen.

Materials and Method

Materials

Silver nitrate was purchased from Sigma Aldrich. Phosphate buffer salts (Na_2HPO_4) and (NaH_2PO_4) were purchased from HIMEDIA. Double distilled water has been used as an aqueous medium for all experiments. All buffers were filtered with 0.2µm filter paper immediately after they were prepared.

Method

1- **Preparation of *Murraya koenigii* bark extract-**

Fresh *M. koenigii* was collected from Kursi Road (Lucknow, India) and used for the preparation of aq. extract. The bark was peeled off from the plant and was cleaned with running tap water, freeze-dried, and bark was crushed in phosphate buffer (pH 7.2) using a pestle and mortar. The resultant extract was centrifuged at 6000 rpm for 10 minutes and then filtered by using Whatman filter paper no.1.

2- **In vitro synthesis of AgNPs-**

In vitro synthesis of AgNPs was done by taking a reaction mixture of 3ml containing 30µl (diluted) of 1mM silver nitrate salt in PBS buffer (pH was 7.2 and it was filtered by 0.2µm filter) and 0.48ml of freshly prepared *Murraya koenigii* bark aqueous extract. This extract was used as a source for the synthesis of AgNPs and served as a reducing agent and also provide stability to particles. On completion of the reaction, the synthesized Silver Nanoparticles were centrifuged for 5 minutes at 5000rpm. The supernatant and the pellet were separated with the help of a 0.2µm filter. This was followed by the characterization of AgNPs using different physiochemical techniques.

Antibacterial activity of synthesized silver Nanoparticles- Preparation of growth media-

For the preparation of media, 13.3 gm of MHA was taken in 350 ml of distilled water in a conical flask and was sterilized for 15-20 minutes in the autoclave.

- **Preparation of bacterial culture plates-**

  The media was poured into the 4 culture plates to prepare the cultures of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Salmonella spp.* and were kept at room temperature for solidifying (all the steps were taken out in aseptic conditions i.e., Laminar Air Flow)
- The 4 wells were created into the plates to pour the antibiotic sample (50µl) and

Fresh plant extract (80µl) in 2 wells separately, Synthesized Silver Nanoparticles (80µl) in 3rd well and 4th well was left controlled.

- The antibiotic and Fresh Plant Extract was poured to check their efficacy when compared to Synthesized Silver Nanoparticles. (All the steps were carried out in Laminar Air Flow)
- The plates were kept in the incubator for 24 hours at 37°C.

Results

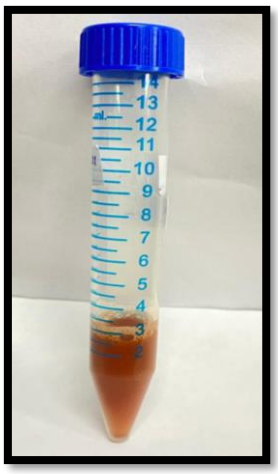

Fig. 6. Synthesized Silver Nanoparticles.

Characterization of Silver Nanoparticles-
UV/VIS spectroscopy

UV/Vis spectrophotometric measurements were performed on a Shimadzu dual-beam spectrophotometer operated at a resolution of 1nm in the quartz cuvette. Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the received color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence and deals with transitions from an excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

To observe the optical property of biosynthesized silver nanoparticles, samples were periodically analyzed for UV- vis spectroscopic studies at room temperature operated at a resolution of 1nm between 250and 800nm ranges.

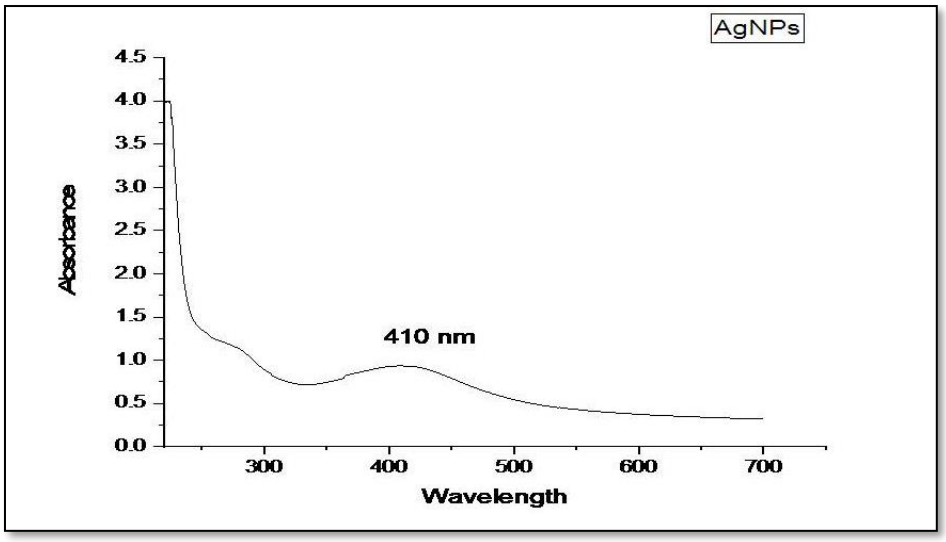

Fig. 7. UV-Visible Spectroscopy of AgNPs shows a distinct and broad absorption band at 410 nm.

The synthesis of silver nanoparticles by the reduction of aqueous metal ions during the exposure of alkalinized *M. koenigii* bark extract was easily monitored by using UV−vis spectrophotometry. In this study, the formation of AgNPs was monitored by measuring the UV−vis spectra at different time intervals (figure 2). As the time increased, the intensity of the absorbance increased, indicating an increase in the amount of AgNPs produced by the mixture. A UV absorption spectrometric analysis of the AgNPs showed absorbance spectra at 435 nm, suggesting the bioreduction of silver nitrate into silver nanoparticles. The broad absorption band at 410 nm is due to surface plasmon resonance typical of silver nanoparticles.

DLS (Dynamic Light Scattering)

The thin electric dipole layer of the solvent adheres to the surface of a dispersed nanoparticle when it moves through a liquid medium; therefore, the hydrodynamic diameter estimated by DLS provides us information about the inorganic core along with coating material and the solvent layer attached to the particle.

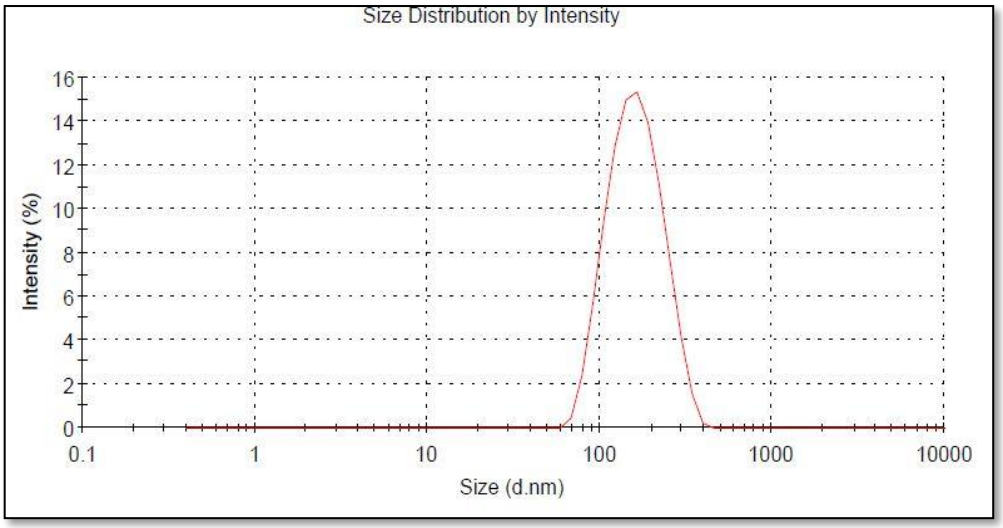

Fig. 8. Dynamic Light Scattering graph shows the average value (65 d.nm.) of SNPs.

Zeta Potential

The determination of Zeta Potential is considered an effective, simplest, and most straightforward method to predict the stability and understand the surface properties of the nanoparticles. Information regarding the concentration, distribution, exposure, or shielding of charged moieties, ionization, and adsorption could be drawn from the analysis of zeta potential.

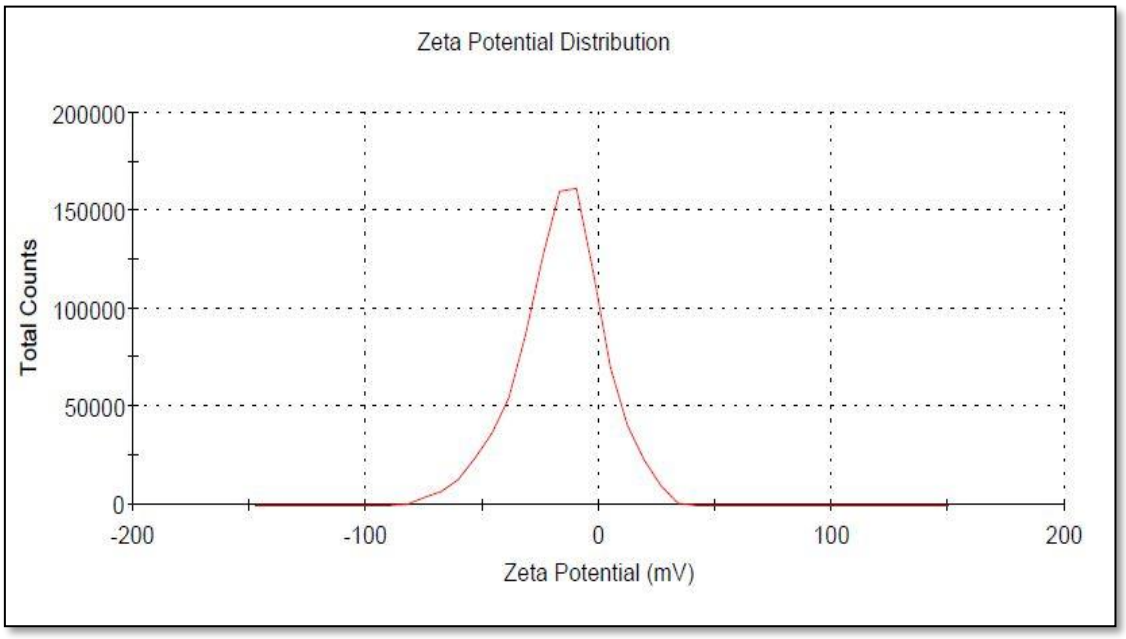

Fig. 8. Zeta potential graph shows a stability peak at -17mV.

FTIR

The FTIR analysis of synthesized AgNPs depicts an existing peak focused at 1643.24 cm^{-1}, i.e., uniqueness of amide C=O groups. A medium and wide shoulder for the amide I linkage and amide II band was observed at 1537.02 cm^{-1}. N–H twist and carboxyl stretch in the protein amide bond were found to be responsible for the presence of amides bands I and II that are capped or surface-modified on AgNPs. The N–H stretch vibration peak was observed at 3296.5 cm^{-1}; however, this vibration is susceptible to hydrogen bond strength with no dependence on backbone conformation. Moreover, the alcohol and ether group (C–O–C/C–OH) C–O stretch along with (aliphatic amine) the C–N stretch vibration, showed a peak at 1081.8 cm^{-1}. The alkynes C≡C stretched vibration because numerous secondary metabolites showed a peak at 2127.39 cm^{-1}. Peaks at 3756.013 and 3868.76 cm^{-1} were observed for free (O–H) hydroxyl on the terminus.

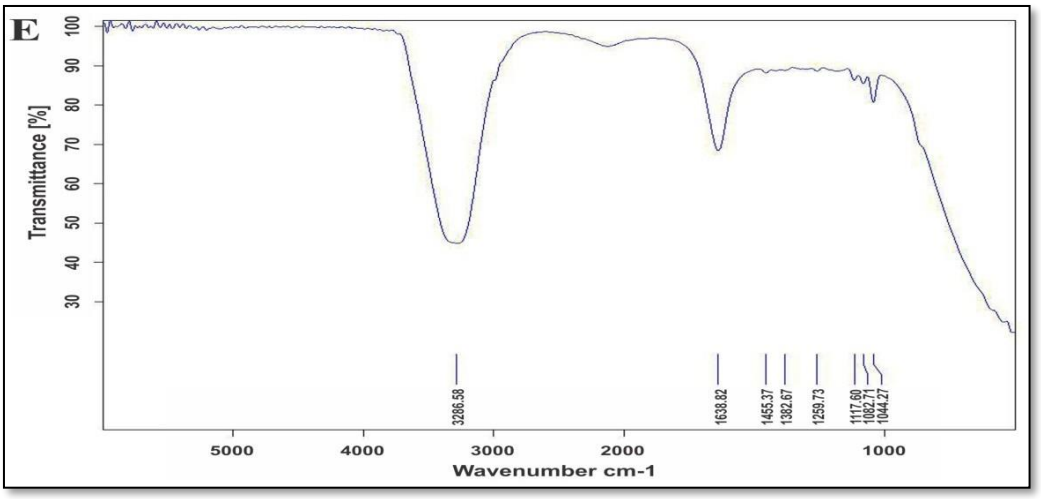

Fig. 9. FTIR spectroscopy.

Transmission electron microscopy

The high-resolution image was acquired using the transmission electron microscope (TEM), which confirmed the average size of AgNPs as 26 ± 2 nm using a Gatan digital micrograph and showed the spherical form of AgNPs. The TEM

micrographs did the average size of AgNPs as 26 ± 2 nm using a Gatan digital micrograph and showed the spherical form of AgNPs. The TEM micrographs did not expose the agglomeration of the as-synthesized AgNPs. Not expose the agglomeration of the as-synthesized AgNPs.(TEM), which confirmed.

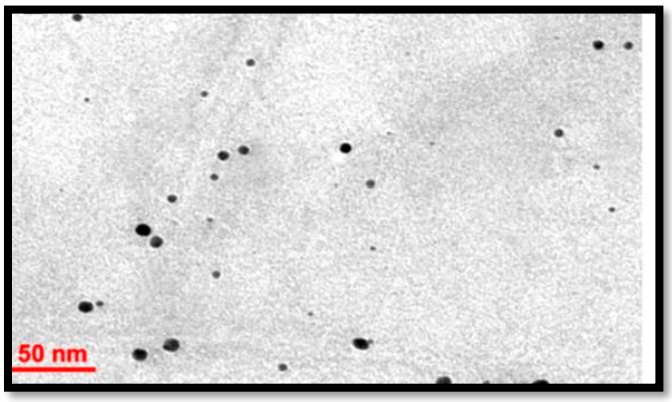

Fig. 10. TEM Analysis shows the average size of AgNPs is 26nm.

Antibacterial screening

The antibacterial activity of *M. koenigii* bark extract and MK-AgNPs was investigated using the disc diffusion method against gram-negative *(E. coli)* and gram-positive (*Staphylococcus aureus*) bacterial strains.

Maximum efficacy against *E. coli and Staphylococcus aureus was reported at a concentration of* 50µg/well crude bark extract of *M. koenigii* with inhibitory zones of 16±0.1 and 11±0.1. Furthermore, the measured zone of inhibition in the instance of levofloxacin (50µg/well) 19±0.1 and 13±0.9 against *E. coli and Staphylococcus aureus* all are resistant to this antibiotic. However, a similar concentration (50µg/well) of AgNPs showed a considerably high zone of inhibition when compared to *M. koenigii* bark extract and levofloxacin. The zone of inhibition was formed by MK-AgNPs 25±0.9 and 27±0.9 against *E. coli and Staphylococcus aureus respectively.*

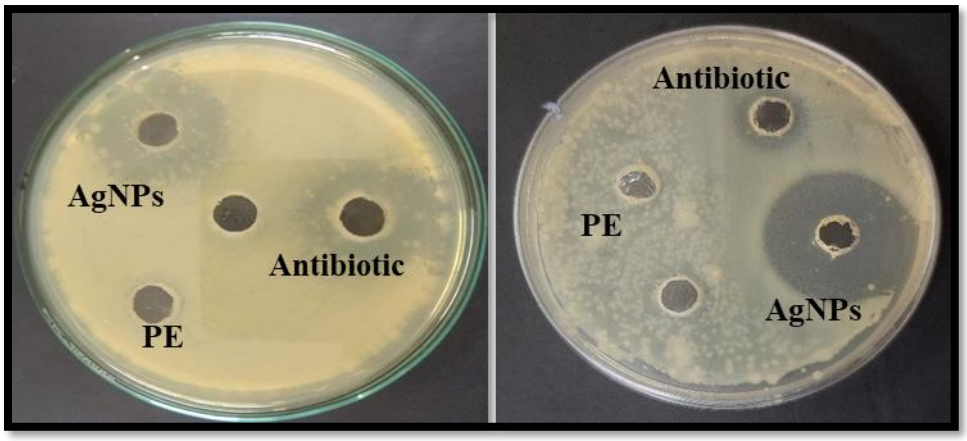

Fig. 11. Shows the antibacterial activity of AgNPs against (A) E. coli and (B) Staphylococcus aureus. (Well diffusion method).

Table showing zone of inhibition of AgNPs.

S.No.	Bacterial strain	Zone of inhibition (mm)	
		AgNPs 50µg/ml	**Antibiotic (levofloxacin)**
1	*Staphylococcus aureus*	**31**	**20**
2.	*Pseudomonas aeruginosa*	**29**	**25**

Minimal Inhibitory Concentration (MIC) Determination

To quantify the antibacterial capabilities and determine the MIC of the pure *M. koenigii*, MK-AgNPs, and levofloxacin (used as control), the test was performed on a sterile 96-well plate, as described in many studies. The crude *M. koenigii* bark extract and MK-AgNPs were serially diluted in a nourishing broth. Then, each well of 96-well plates was incubated for 24 h at 37 ◦C with a standardized suspension of bacteria (103 cells/mL). After 24 h, an ELISA reader [(Microplate Reader (BIORAD-680)] was used to read the plate at 625 nm, and subsequently, the reading was recorded for further use.

The MIC is the lowest concentration of *M. koenigii* bark extract and MK-AgNPs that completely inhibit bacterial growth, and MIC_{50} is the concentration of plant extract and MK-AgNPs that inhibit 50% of the bacterial population. The MIC_{50} of *M. koenigii* bark extract and MK-AgNPs against several Gram-negative and Gram-positive

bacterial strains were recorded. However, levofloxacin was used as a standard antibiotic during the experiment. The quantified MIC_{50} values were *E. coli* 0.91 mg/mL and Staphylococcus aureus, 0.61 mg/ml.

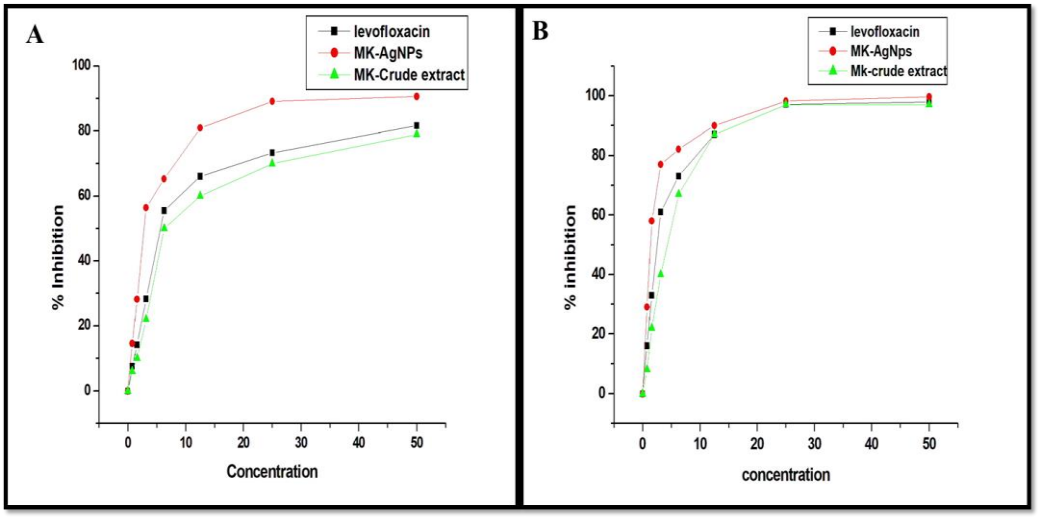

Fig. 13. Minimum Inhibitory Concentration (8µg/ml) of pure MK-extract aqueous extract and MKAgNPs against (A) E. coli and (B) Staphylococcus aureus (96 well plate method).

Discussion and conclusion

Discussion and conclusion

M. koenigii bark extract was utilized as a reducing and stabilizing agent in this work. The synthesis of AgNPs is thought to be triggered by *M. koenigii* aqueous bark extract's reducing enzymes and capping agents, such as secondary metabolites, which work together to decrease silver nitrate. The color of the synthesized AgNPs was Ruby Red color which is the characteristic feature of AgNPs. According to Mie Theory, silver shows resonance known as Plasmons in the UV-visible spectrum. These resonances are formed by the interaction of electromagnetic waves and electrons at the surface of AgNPs. This resonance characteristic of AgNPs can be observed by spectroscopy.

The antibacterial activity of AgNPs was determined against four species of Gram-negative e pathogens: *E. coli* and *Staphylococcus aureus*. The results of the disk diffusion test, of the AgNPs are summarized in Table. For the disk diffusion test, the presence of a clear zone around the AgNPs disk suggests that the AgNPs possessed antibacterial activity which can inhibit the growth of the pathogens. The visible clear zone produced by AgNPs against four different species of Gram-negative and gram-positive bacteria is shown in Figure. 11.

Conclusion

A simple, one-step green approach was developed for the synthesis of AgNPs using, *M. koenigii* bark extracts the plant extract acts as both a reducing and stabilizing agent. Silver nanoparticles showed significant antibacterial activity against the selected Gram-negative and gram-positive pathogens. Thus, AgNPs might be a good alternative to develop as an antibacterial agent against multidrug-resistant strains of bacteria. The applications of AgNPs may lead to valuable findings in various fields such as medical devices and antimicrobial systems.

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for the synthesis of metallic nanoparticles. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physicochemical properties. The silver nanoparticles synthesized from alkalinized *M. koenigii* bark extract by a bio-reduction method exhibit all the characteristic features of nanoparticles.

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