A DISSERTATION ON

Biosynthesis of Selenium Nanoparticles by using *Piper Betel (PAN)* aqueous leaves extract

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BY

Kahkashan

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M.Sc. Biotechnology (IV semester)

Department of Biosciences

Integral University, Lucknow

UNDER THE SUPERVISION OF

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Kahkashan**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled ***"Biosynthesis of Selenium Nanoparticles by using Piper Betel (PAN) aqueous leaves extract"*** successfully. She 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 her M.Sc. degree. I wish her good luck and a bright future.

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

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IU/DBS/S00192/2022/CIN/221114 **June 2022**

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Kahkashan**, during the months 2 Feb to 2 June 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the script of the thesis has been written by the candidate herself. The thesis entitled ***"Biosynthesis of Selenium Nanoparticles by using Piper Betel (PAN) aqueous leaves extract"*** 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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Kahkashan **Date**

List of abbreviations

Se-NPs	Selenium nanoparticles
PBS	Phosphate buffer
M	Molarity
mM	Milli Molar
DLS	Dynamic Light Scattering
OD	Optical density
M deg	Milli degree
SPR	Surface Plasma Resonance
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
UV-Vis	Ultraviolet Visible Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
ZP	Zeta potential
nm	Nano meter

Introduction

Nanotechnology 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 100 nanometers 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 tolerability, 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. This article presents an overview of nanotechnology for the biologist and discusses “nanotech” strategies and constructs that have already demonstrated in vitro and in vivo efficacy.

By providing constructs capable of combining multiple functionalities into a single nanoscale entity, nanotechnology also offers the opportunity to monitor and detect molecular and cellular changes associated with disease states. Given this multifunctional capability, one can imagine building a nanoparticle that can target a specific tissue or cell type, delivering a contrast agent that allows for noninvasive imaging and a therapeutic payload to the target. A nanoparticle might even contain a reporter, such as an apoptotic marker, which signals that the payload has been delivered and is having the desired therapeutic effect. Such combinatorial nanostructures may eventually provide the means to achieve “personalized medicine” by tailoring drug delivery to individual responses. Although this may seem futuristic, several groups have already created multifunctional nanodevices and are testing them *in vitro* and *in vivo* systems **[8].**

Research and product developments in the area of nanotechnology have steadily increased especially due to the new, beneficial properties of nanomaterials. Nanotechnology as a cross-cutting technology, nowadays used in electrical devices, in construction and composite materials, as catalysts, and as antibacterial coatings, is more and more present in workplaces as well as consumer products. This steady increase is accompanied by larger production, handling, and processing facilities for nanostructured materials and a higher tonnage of nanomaterials **[9].**

Nanoparticles

Nanoparticles are particles of sizes ranging from 1 to 100*nm* 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].

Review of literature

Nanotechnology is a known field of research since the last century. Since ‘‘nanotechnology” was presented by Nobel laureate Richard P. Feynman during his well famous 1959 lecture ‘‘There’s Plenty of Room at the Bottom” (Feynman, 1960), there have been made various revolutionary developments in the field of nanotechnology. Nanotechnology produced materials of various types at the nanoscale level. Nanoparticles (NPs) are a wide class of materials that include particulate substances, which have one dimension less than 100 nm at least (Laurent et al., 2010). Depending on the overall shape these materials can be 0D, 1D, 2D, or 3D (Tiwari et al., 2012). The importance of these materials was realized when researchers found that size can influence the physicochemical properties of a substance e.g. the optical properties. A 20*nm* gold (Au), platinum (Pt), silver (Ag), and palladium (Pd) NPs have characteristic wine red color, yellowish gray, black and dark black colors **[1].**

Nanoparticles serve various industrial and domestic purposes which are reflected in their steadily increasing production volume. This economic success comes along with their presence in the environment and the risk of potentially adverse effects on natural systems. Over the last decade, substantial progress regarding the understanding of sources, fate, and effects of nanoparticles has been made. Predictions of environmental concentrations based on modeling approaches could recently be confirmed by measured concentrations in the field. Nonetheless, analytical techniques are, as covered elsewhere, still under development to more efficiently and reliably characterize and quantify nanoparticles, as well as to detect them in complex environmental matrixes. Simultaneously, the effects of nanoparticles on aquatic and terrestrial systems have received increasing attention. While the debate on the relevance of nanoparticle-released metal ions for their toxicity is still ongoing, it is a re-occurring phenomenon that inert nanoparticles can interact with biota through physical pathways such as biological surface coating. This among others interferes with the growth and behavior of exposed organisms. Moreover, co-occurring contaminants interact with nanoparticles. There is multiple evidence suggesting nanoparticles as a sink for organic and inorganic co-contaminants. On the other hand, in the presence of nanoparticles, repeatedly an elevated effect on the test species induced by the co-contaminants has been reported. In this paper, we highlight recent achievements in the field of nano-ecotoxicology in both aquatic and terrestrial systems but also refer to substantial gaps that require further attention in the future **[2].**

These NPs showed characteristic colors and properties with variations in size and shape, which can be utilized in bioimaging applications (Dreaden et al., 2012). The color of the solution changes due to variation in aspect ratio, nanoshell thickness, and % gold concentration. The alteration of any of the above-discussed factors influences the absorption properties of the NPs and hence different absorption colors are observed. NPs are not simple molecules themselves and therefore composed of three layers i.e. (a) The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants, and polymers. (b) The shell layer, which is a chemically different material from the core in all aspects, and (c) The core, which is essentially the central portion of the NP and usually refers to the NP itself (Shin et al., 2016).

Table 1: Periodical development in nanotechnology. **[34]**

1989	IBM logo was made with individual atoms
1991	S. Iijima discovered 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

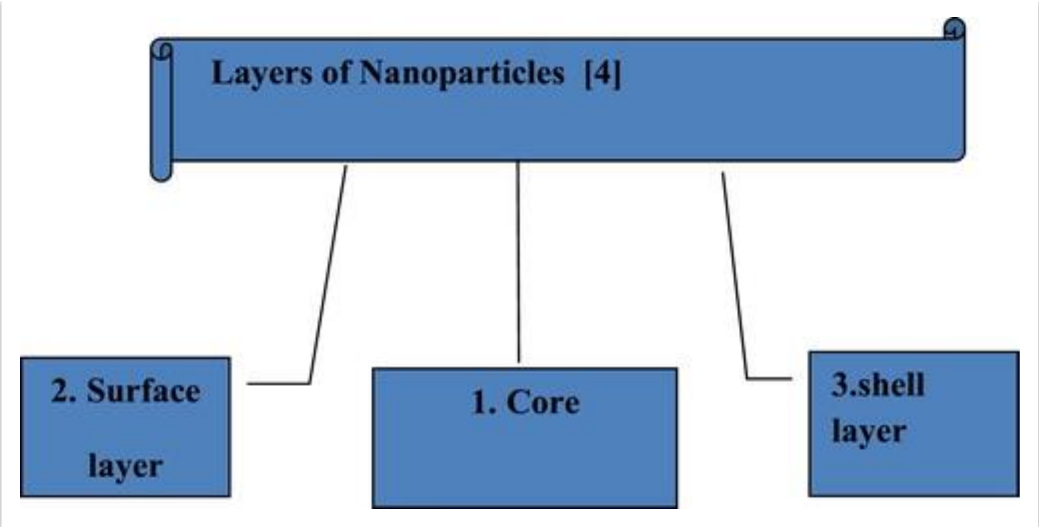

Fig. 1. A different layer of nanoparticles

History of Nanotechnology

The development in the field of nanotechnology started in 1958 and the various stages of development have been summarized in Table

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	The first book on nanotechnology Engines of Creation published by K. Eric Drexler,

Classification of NPs

NPs are broadly divided into various categories depending on their morphology, size, and chemical properties. Based on physical and chemical characteristics, some of the well-known classes of NPs. Carbon-based NPs fullerenes and carbon nanotubes (CNTs) represent two major classes of carbon-based NPs. Fullerenes contain nanomaterial that is made of the globular hollow cage such as allotropic forms of carbon.

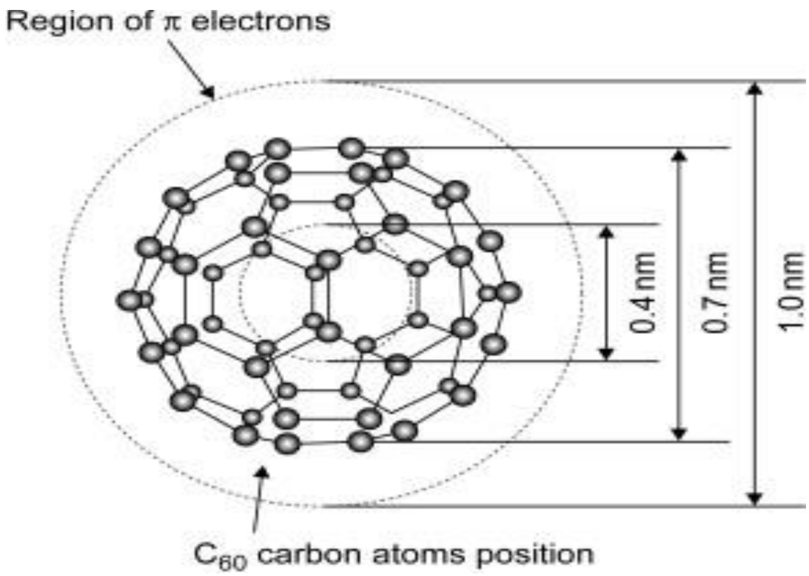

Fig. 2. A figure depicting the structure of a fullerene nanoparticle.

They have created noteworthy commercial interest. Due to their electrical conductivity, high strength, structure, electron affinity, and versatility (Astefanei et al., 2015). These materials possess arranged pentagonal and hexagonal carbon units, while each carbon is sp2 hybridized. some of the well-known fullerenes consist of C60 and C70 with a diameter of 7.114 and 7.648 nm, respectively. CNTs are elongated, tubular structures, 1–2 nm in diameter (Ibrahim, 2013). These can be predicted as metallic or semiconducting reliant on their diameter telicity (Aqel et al., 2012). These are structurally resembling graphite sheets rolling upon themselves. The rolled sheets can be single, double, or many walls and therefore they are named single-walled (SWNTs), double-walled (DWNTs), or multi-walled carbon nanotubes (MWNTs), respectively. They are widely synthesized by deposition of carbon precursors especially the atomic carbons, vaporized from graphite by laser or by electric arc onto metal particles. Lately, they have been synthesized via the chemical vapor deposition (CVD) technique (Elliott et al., 2013). Due to their unique physical, chemical, and mechanical characteristics, these materials are not only used in pristine form but also nanocomposites for many commercial applications such as fillers (Saeed and Khan, 2016, 2014), efficient gas adsorbents for environmental remediation (Ngoy et al., 2014), and as a support medium for different inorganic and organic catalysts (Mabena et al., 2011).

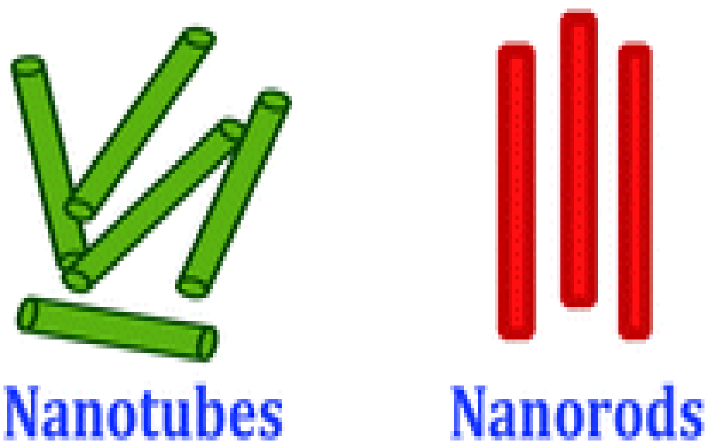

Fig. 3. A figure depicting the shape of nanorods and nanotubes.

Metal NPs

They are purely made of metal precursors. Due to well-known localized surface plasmon resonance (LSPR) characteristics, these NPs possess unique optoelectrical properties. NPs of the alkali and noble metals i.e. Cu, Ag, and Au have a broad absorption band in the visible zone of the electromagnetic solar spectrum. The facet, size, and shape-controlled synthesis of metal NPs are important in present-day cutting-edge materials (Dreaden et al., 2012). Due to their advanced optical properties, metal NPs find applications in many research areas. Gold NPs coating is widely used for the sampling of SEM, to enhance the electronic stream, which helps in obtaining high-quality SEM images. There are many other applications, which are deeply discussed in the applications section of this review.

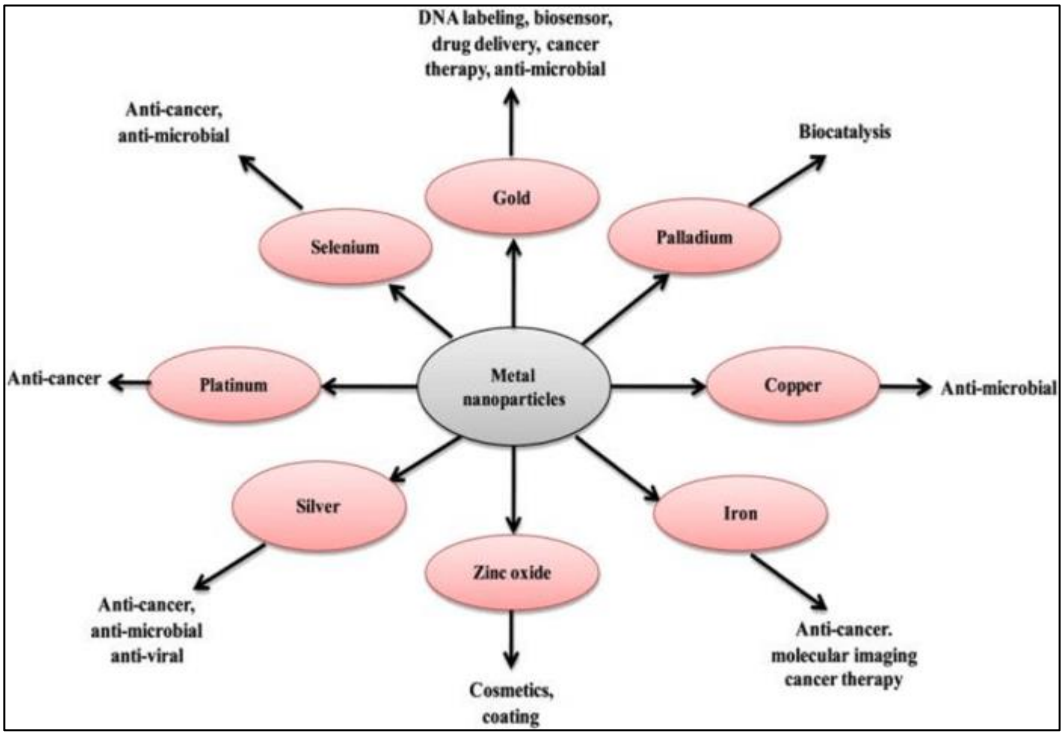

Fig. 4. A figure describing the types of metal nanoparticles and their uses.

Ceramics NPs

Ceramics NPs are inorganic nonmetallic solids, synthesized via heat and successive cooling. They can be found in amorphous, polycrystalline, dense, porous, or hollow forms (Sigmund et al., 2006). Therefore, these NPs are getting the great attention of researchers due to their use in applications such as catalysis, photocatalysis, photodegradation of dyes, and imaging applications. (Thomas et al., 2015). 2.4. Semiconductor NPs Semiconductor materials possess properties between metals and nonmetals and therefore they found various applications in the literature due to this property (Ali et al., 2017; Khan et al., 2017a). Semiconductor NPs possess wide bandgaps and therefore showed significant alteration in their properties with bandgap tuning. Therefore, they are very important materials in photocatalysis, photo optics, and electronic devices (Sun, 2000). As an example, a variety of semiconductor NPs is found exceptionally efficient in water splitting applications, due to their suitable bandgap and band-edge positions (Hisatomi et al., 2014).

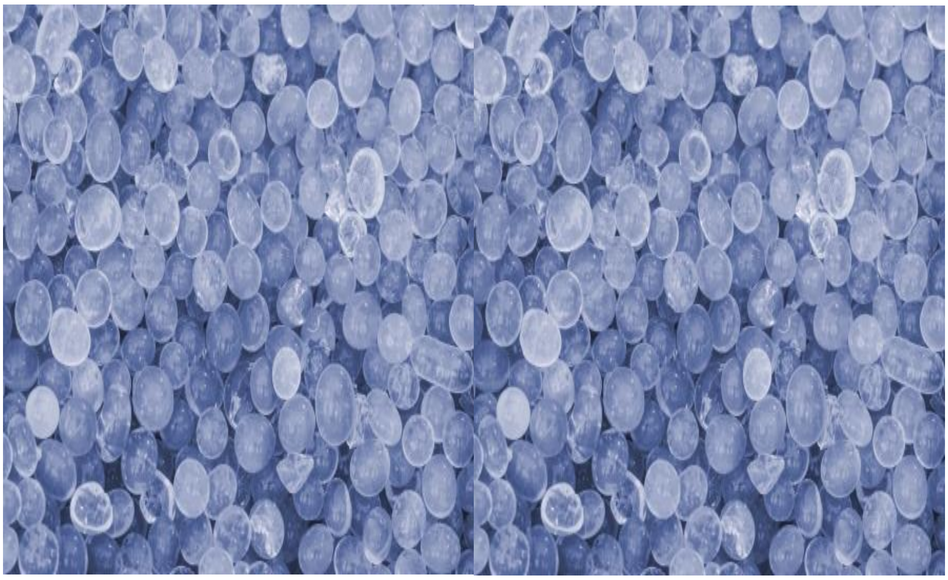

Fig. 5. A figure shows the picture of ceramic nanoparticles.

Polymeric NPs

These are normally organic-based NPs and in the literature, a special term polymer nanoparticle (PNP) collective is used for it. They are mostly nanospheres or nanocapsules shaped (Mansha et al., 2017). The former are matrix particles whose overall mass is generally solid and the other molecules are adsorbed at the outer boundary of the spherical surface. In the latter case the solid mass is encapsulated within the particle completely (Rao and Geckeler, 2011). The PNPs are readily functionalized and thus find bundles of applications in the literature (Abd Ellah and Abouelmagd, 2016; Abouelmagd et al., 2016).

Lipid-based NPs

These NPs contain lipid moieties and are effectively used in many biomedical applications. Generally, a lipid NP is characteristically y spherical with a diameter ranging from 10 to 1000 nm. Like polymeric NPs, lipid NPs possess a solid core made of lipid and a matrix that contains soluble lipophilic molecules. Surfactants or emulsifiers stabilized the external core of these NPs (Rawat et al., 2011). Lipid nanotechnology (Mashaghi et al., 2013) is a special field, which focuses on the designing and synthesis of lipid NPs for various applications such as drug carriers and delivery (Puri et al., 2009) and RNA release in cancer therapy (Gujrati et al., 2014).

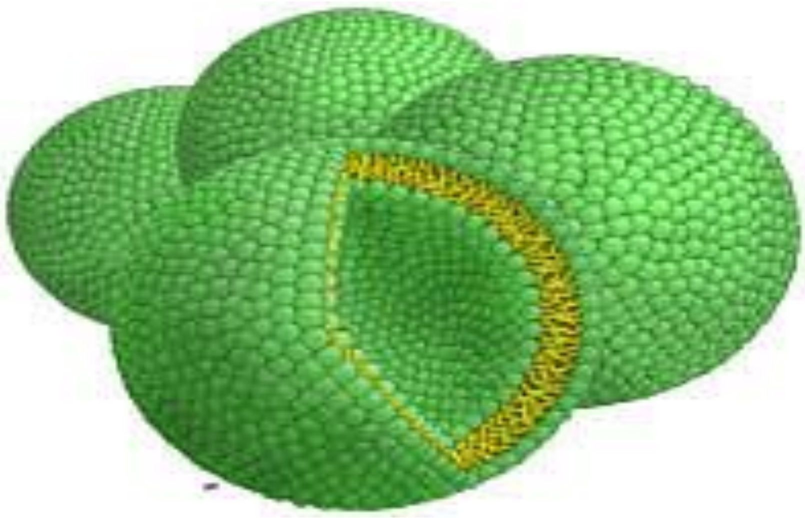

Fig. 6. A Lipid-based nanoparticle**.**

Nanoparticles (NPs) are widely employed in different research areas, ranging from analytical chemistry and environmental science to medicine, agriculture, and the pharmaceutical industry. This is mainly due to the unique characteristics of NPs and the novelty they introduce in such applications. In analytical chemistry, the role of NPs can differ depending on the nature of the steps involved in the analytical process. NPs are probably most useful for detection, but sample preparation has also profited from them. For instance, NPs can advantageously replace conventional sorbents for solid-phase extraction. Moreover, NPs are being increasingly used as stationary phases in gas and liquid chromatography or electrochromatography. In this review, a summary of the classification, synthesis methods, and properties of NPs is given. Moreover, the examples of applications in the different research areas are shortly presented. However, the merits of this work are to present the use of NPs in the analytical chemistry field **[3].**

Methods for the synthesis of nanoparticles

High-throughput NPs with good and controlled quality are desirable for their commercialization in various fields of applications. There are two basic approaches commonly employed to prepare NPs; (1) the top-down approach, where synthesis is initialized with the bulk counterpart that leaches out systematically bit-after-bit leading to the generation of fine NPs. Photolithography, electron beam lithography, milling techniques, anodization, and ion and plasma etching are some of the commonly used top-down methods for the mass production of NPs; (2) bottom-up approach, which involves the coalescence or assembling of atoms and molecules to generate a diverse range of NPs. Examples of bottom-up approaches include self-assembly of monomer/polymer molecules, chemical or electrochemical nanostructural precipitation, sol-gel processing, laser pyrolysis, chemical vapor deposition (CVD), plasma or flame spraying synthesis, and bio-assisted synthesis.[61] In general, NP synthesis methods can be divided into three groups – (1) physical methods, (2) chemical methods, and (3) bio-assisted methods. **[4]**

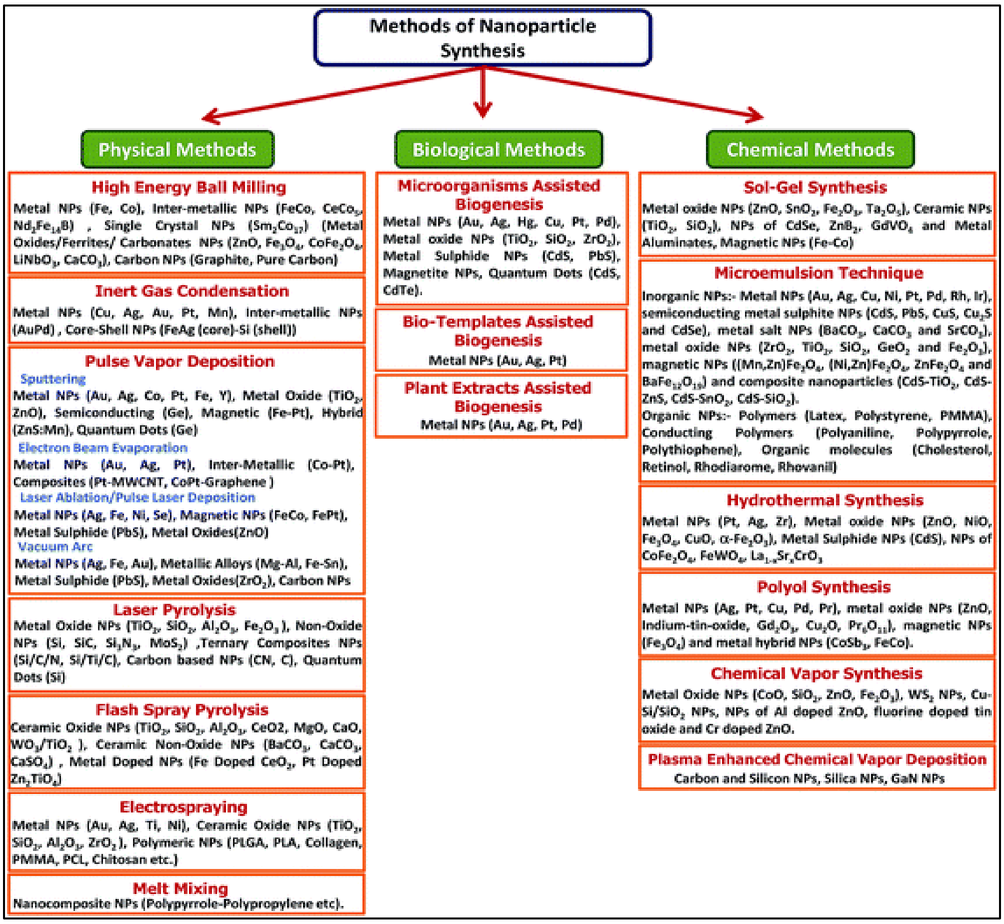

Fig. 7. Overview of different types of synthesis methods to produce a variety of nanoparticles.

Plant extracts for nanoparticles synthesis

Biosynthesis of NPs using plant extracts or plant biomass is one of the very effective, rapid, clean, non-toxic, and eco-friendly methods. This method has been utilized predominantly to synthesize NPs of noble metals, metal oxides, bi-metallic alloys, etc. Yun et al. have adequately demarcated various plant biometabolites that could help in the preparation of NPs based on their valuable role as reducing agents and capping agents. The kinetics of photosynthesis of NPs is comparatively much higher than that of other biosynthesis methods and sometimes equivalent to the rate of chemical routes. Shankar et al. reported the preparation of gold nano triangles by treating the lemongrass leaf extract with the aqueous $AuCl^-$ ions. Similarly, the leaf extract of different plants like Tamarindus indica, Aloe vera, Emblica Officinalis, etc. was reported to be useful for designing Au

NPs. Few-nanometer-sized Pd NPs and Pt NPs were prepared using the extract taken out from various parts of different plants. Shankar et al. also reported highly concentrated Ag NPs obtained from the leaf extract of Azadirachta indica and the fruit extract of Emblica Officinalis. Li et al. and Leela et al. used the leaf extract of Aloe vera, Capsicum annuum and Helianthus annuus for the efficient synthesis of Ag NPs. Extracellular production of Cu NPs was carried out using stem latex of Euphorbia nivulia (medicinal plant) which were found to be stabilized by the peptides and terpenoids present within the latex. In a very interesting report, Seraphin et al. demonstrated the synthesis of In O_3 NPs (5–50 nm) using plant extract from Aloe vera. Furthermore, Hou et al., synthesized wurtzite ZnO NPs from the Zn-hyper-accumulator plant Sedum al-fredii with a mean size of 53.7 nm. Ascencio et al. reported the bio-assisted synthesis of iron-oxide NPs by using the biomass of the Medicago sativa (alfalfa) plant. Glutathione, an antioxidant tripeptide in plants, animals, fungi, and archaea, was reported to design well-ordered aggregates of Au NPs by favoring the inter-particle interactions. Kundu and Nithiyanantham also demonstrated the in situ fabrication of shape-selective silver nanostructures using curcumin as stabilizing and reducing agent **[4].**

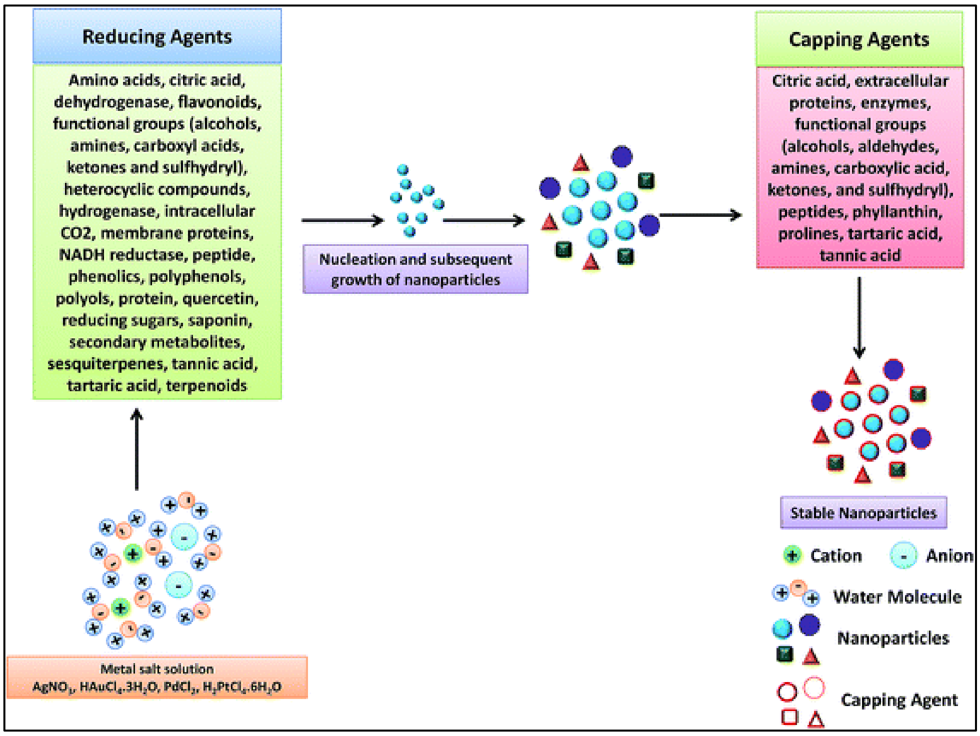

Fig. 8. The mechanism for the biogenic synthesis of metal NPs using plant extract.

Nowadays, biological synthesis is an attractive alternative to traditional methods (physical and chemical approaches) for the production of nanoparticles. The bioreduction synthesis involves the use of different biological entities, such as plant extracts, bacteria, fungi, and yeasts. Bioreduction using plant extracts. The plant extracts contain bioactive alkaloids, phenolic acids, polyphenols, proteins, sugars, and terpenoids which confer the ability to reduce the metal ions to nanoparticles. The plants were considered an ecological way for nanoparticle synthesis, especially for those materials which have to be used in the biomedical field. Bioreduction using microorganisms. Microorganisms can survive and grow in high concentrations of toxic metals due to their chemical detoxification potential, as well as their energy-dependent efflux in the cell by membrane protein, which functions either as ATPase or as chemo-osmotic or proton anti-transporter agents. Biosynthesis is a phenomenon that occurs through a biological or enzymatic reaction. The exact mechanism for the synthesis of nanoparticles using biological agents has not yet been perfected, as the different biological agents are using different mechanisms with different metals, and there are also different biomolecular responses for nanoparticle synthesis **[5].**

There are different methods for the synthesis of nanoparticles and these methods are divided into two main classes.

Top-down synthesis

In this synthesis, the destructive method is used. The larger molecule (bulk material) decomposed into a smaller molecule and then these smaller molecules transform into the nanoparticles. Grinding or milling, physical vapor deposition, and other destructive approaches are an example of Top-down synthesis. Coconut Shell nanoparticles are synthesized by using this method. Coconut Shell nanoparticles were synthesized using the milling method and raw coconut shells are finely ground for different intervals of time by using the planetary mill and ceramic ball. They observed that the overall size of nanoparticles was affected by milling time through different characterization techniques. It was observed that the crystal-lite size of nanoparticles decreased with an increase in milling time as calculated by using the Scherrer equation. X-ray indicates that the particle size decrease with time. SEM result also agreed with the X.ray pattern. One study showed the synthesis of magnetite having a spherical shape from iron oxide by using the top-down method. The top-down approach was used to synthesize colloidal carbon spherical particles having a size range from 20 to 50 nm. The synthesis method depended on the chemical adsorption of polyoxometalates on the surface of interfacial carbon.

Bottom-up method

The bottom-up method is also known as the constructive method. It is the reverse of the top-down method. In this method, nanoparticles are formed from relatively simpler substances. The bottom-up method includes Chemical vapor deposition (CVD), Sol-gel, spinning, pyrolysis, and biological synthesis.

Chemical vapor deposition (CVD) method

It is the method in which a thin film of gaseous reactant is deposited on the substrate. The deposited thin film is carried out in a reaction chamber. A chemical reaction takes place by combining gas in contact with a heated substrate. Thin-film of product is produced on the surface of the substrate as a result of this reaction. This thin film is recovered and used. Hard, strong, uniform, and highly pure nanoparticles are advantages of the CVD method. The requirement for special apparatus and the formation of highly toxic gaseous as by-products are disadvantages of the CVD method.

Sol-gel method

The sol-gel method is a combination of two words sol and gel. Sol is a colloid formed from solid particles suspended particles in a continuous liquid. The gel is a solid macro-molecule that is dissolved in a solvent. Due to its simplicity, the sol-gel method is the most preferred bottom-up method for the synthesis of nanoparticles. It is the method in which a suitable chemical solution act as a precursor. The typical precursors are metal oxide and chloride used in the sol-gel method. By using different methods such as stirring, sonication, and shaking, the precursor is dispersed in the host liquid. The resultant solution consists of a solid phase and liquid which is separated by using different techniques such as filtration, sedimentation, and centrifugation to recover the nanoparticles **[7].**

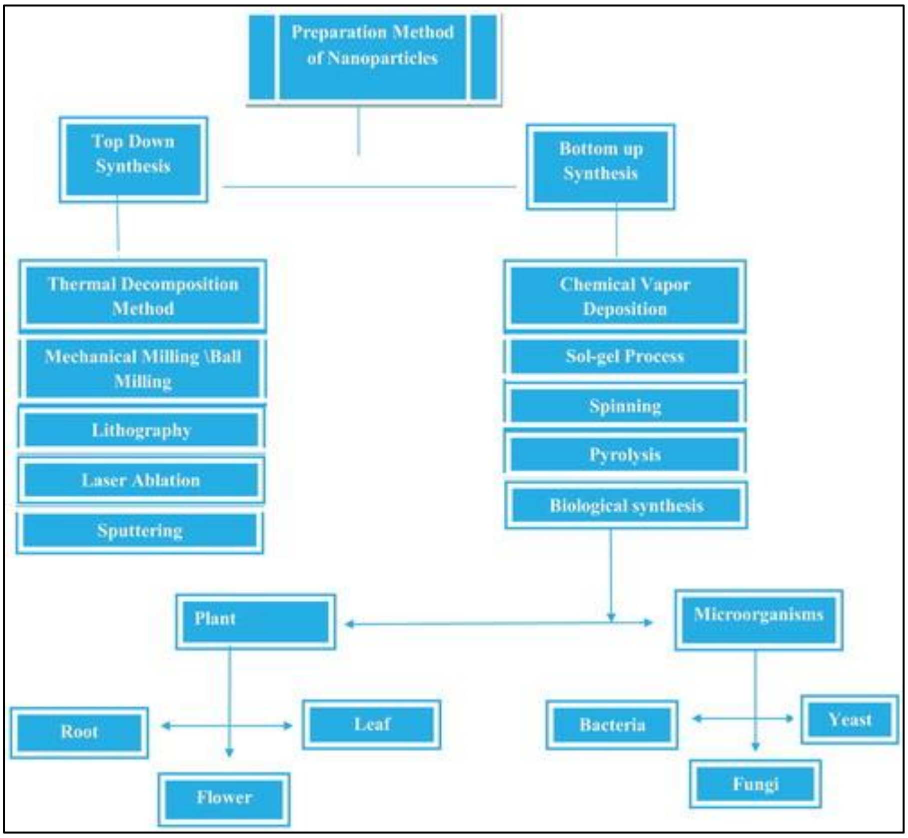

Fig. 9. Methods of nanoparticles synthesis

Selenium nanoparticles (SeNPs)

SNPs exhibit attractive anticancer activity and reduced toxicity concerns compared to different Se species. SeNPs have been used in many disease conditions including cancer, diabetes, inflammatory disorders, liver fibrosis, and drug-induced toxicities SeNPs scavenge the free radicals *in vitro* in a size-dependent manner (5 nm–200 nm). Bo Huang et al. showed that small-sized (5–15 nm) SeNPs have better free radical scavenging capacity and prevented the oxidation of DNA. The SeNPs showed superior effects at <0.5 mM concentration compared to free Na_2SeO_3 which exhibited IC_{50} >2.5 mM. SeNPs show better bioavailability, and biological activity compared with inorganic and organic Se compounds. However, poor cellular intake is the main drawback of SeNPs. Significant attempts have been made to overcome this problem by conjugation of targeting ligands on the exterior surface of nanoparticles. This provides a beneficial platform for anticancer therapy. Surface capping agents control the size, and stability, improve the cancer selectivity, enhance cellular uptake and also improve the bioavailability and biological activity of SeNPs. The introduction of amphoteric ligands such as polyethylene glycol (PEG) has been shown to substantially assist in nanoparticle synthesis. In another study, SeNPs were conjugated with a custom synthesized cyclic peptide which showed improved penetrability in the SK-OV-3 ovarian adenocarcinoma cell line. Thus, SeNPs can be potentially used as nanosized delivery tools for differentially charged biomolecules and anticancer drugs as well **[10].**

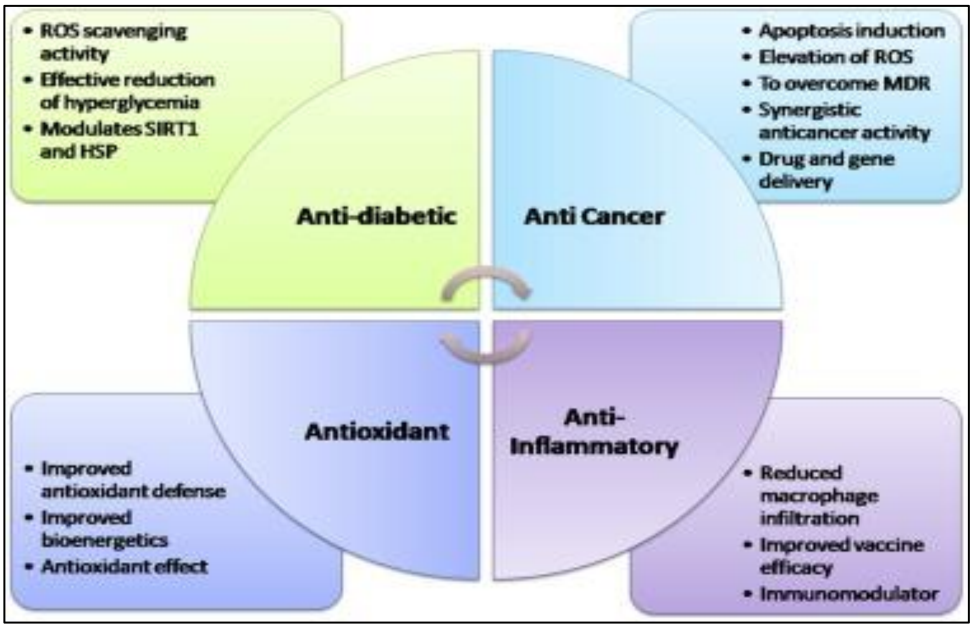

Fig. 10. A figure showing therapeutic applications of selenium nanoparticles (SeNPs). SeNPs possess various therapeutic benefits including anticancer, antioxidant, anti-inflammatory, and anti-diabetic action.

A variety of microorganisms, enzymes, and fungi, besides plant extracts, have been used to synthesize Se nanoparticles of different sizes and morphology. Se itself is used in rectifiers, solar cells, photocopiers, and semiconductors. In addition, they exhibit biological activity owing to their interaction with the proteins and other biomolecules present in the bacterial cells and plant extracts, containing functional groups such as ›NH, C = O, COO, and C-N [9]. Se-nanobelts have been synthesized on large scale with an approximate diameter of 80 *nm* and length up to 5 μm. Se exists in many crystalline and amorphous forms but the shape, size, and structure of the nanoparticles depend on the concentration, temperature, nature of biomolecules, and pH of the reaction mixture. The properties of Se nanoparticles vary with size and shape, for instance, Se nanospheres have high biological activity and low toxicity while Se nanowires of t-Se have high photoconductivity **[11].**

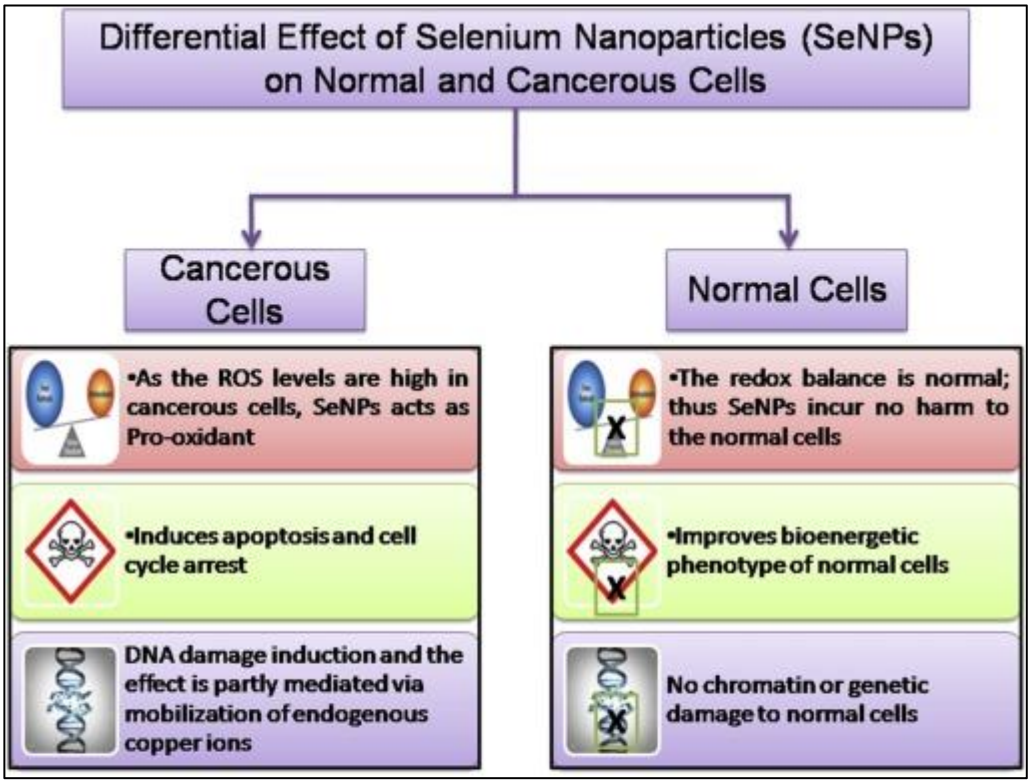

Fig. 11. A figure shows the differential activity of SeNPs on cancer cells and normal cells. SeNPs show prooxidant behavior inside malignant cells owing to the distinct osmotic and redox state of cancerous cells**.**

Applications of Nanotechnology

a) 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 nanodevices 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.

b) 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.

c) 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].**

d) **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.

e) **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 a 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)

f) **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]**

Piper betel leaves

P. betel Linn (Family: Piperaceae) leaves are widely used as a post-meal mouth freshener, and the crop is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian countries. The leaves of this plant are economically and medicinally important and have been traditionally used in India, and China. In Thailand, leaves are used to prevent oral malodor since it has antibacterial activity against obligate oral anaerobes responsible for halitosis [8].

Piper betel (L) is a popular medicinal plant in Asia. Plant leaves have been used as a traditional medicine to treat various health conditions. It is highly abundant and inexpensive, therefore promoting further research and industrialization development, including in the food and pharmaceutical industries. Articles published from 2010 to 2020 were reviewed in detail to show recent updates on the antibacterial and antifungal properties of betel leaves. This current review showed that betel leaf extract, essential oil, preparations, and isolates could inhibit microbial growth and kill various Gram-negative and Gram-positive bacteria as well as fungal species, including those that are multidrug-resistant and cause serious infectious diseases. *P. betel* leaves displayed high efficiency on Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-positive bacteria such as *Staphylococcus aureus*, and *Candida albicans*. The ratio of MBC/MIC indicated bactericidal and bacteriostatic effects of *P. betel* leaves, while MFC/MIC values showed fungicidal and fungistatic effects. This review also provides a list of phytochemical compounds in betel leaves extracts and essential oils, safety profiles, and value-added products of betel leaves. Some studies also showed that the combination of betel leaf extract and essential oil with antibiotics (streptomycin, chloramphenicol, and gentamicin) could provide potentiating antibacterial properties **[12].**

Aqueous extracts of *P. betel* have also been shown to reduce the adherence of early dental plaque bacteria [9]. The leaves of *P*. *betel* have a strong pungent and aromatic flavor and are used as a mouth freshener, in wound healing [10], as a digestive and pancreatic lipase stimulant [11], antioxidant [12, 13], antifungal, antibacterial [1, 14–16], anti-inflammatory, bioprotective [17], and antidiabetic [18] agent. In Chinese folk medicine, it is used for curing wind-cold cough, bronchial asthma, rheumatism, stomachalgia, and pregnancy edema [19]. *P. betel* leaves contain a significant amount of antioxidants such as hydroxychavicol, eugenol, ascorbic acid, and β-carotene [20]. We have successfully synthesized AuNPs by hypothesizing that the presence of strong antioxidants and flavonoids would assist in the reduction of gold ions to AuNPs. To aid this process, we applied microwave (MW) irradiation, which has the advantages of homogeneous heating that directly influence the nucleation process of AuNPs synthesis [21, 22].

Characterization of NPs

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 mentioned below.

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.

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.

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.

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 are 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

- Green synthesis of SeNPs by Piper Betel aqueous leaves extract.
- Characterization of bio-synthesized Piper Betel-SeNPs by using various physical techniques.
- Comparative Antibacterial potential analysis of SeNPs, aqueous leaf extract and Levofloxacin against Pseudomonas aeruginosa, Staphylococcus aureus.

Materials and method

Materials

Selenium oxide (SeO_2) 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. Microbiological media and ingredients were purchased from Himedia, India. All solvents and chemicals were of analytical grade and used as obtained from Merck and Sigma–Aldrich (St. Louis, MO, USA).

Method

Preparation of Piper beetle leaf extract

Fresh Piper beetle leaf was collected from Lucknow, India, and used for the preparation of aq. extract. The leaf was cleaned with running tap water, and the leaf 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.

In vitro synthesis of SNPs

In vitro synthesis of SNPs was done by taking a reaction mixture of 3ml containing 30µl (diluted) of 1mM HAuCl4 salt in PBS buffer (pH was 7.2 and it was filtered by 0.2µm filter) and 0.48 ml of freshly prepared Piper beetle leaf extract. This extract was used as a source for the synthesis of SNPs and served as a reducing agent and also provide stability to particles. The extract reduces SeO_2 anions which were further reduced to form monodispersed, spherical selenium nanoparticles of different sizes. On completion of the reaction, the synthesized selenium nanoparticles were centrifuged for 5 minutes at 5000rpm. The supernatant and the pellet were separated with the help of a 0.2 µm filter. This was followed by the characterization of SNPs using the technique UV-vis Spectroscopy.

Antibacterial activity of synthesized selenium Nanoparticle

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 and Staphylococcus aureus,.* 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 SeNPs (80µl) in the 3rd well and 4th well were left controlled.
- The antibiotic and Fresh Plant Extract was poured to check their efficacy when compared to synthesized selenium 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

Results

Fig. 12. Synthesized selenium nanoparticles.

Characterization of Selenium Nanoparticles

Characterization was done by using UV- visible spectroscopy. The absorption spectra of SeNPs were recorded on Shimadzu Dual Beam Spectrophotometer (model UV–1601PC) in the wavelength range of 200-800 nm in Quartz Cuvette of 1 cm Path Length.

UV/Vis SPECTROSCOPY

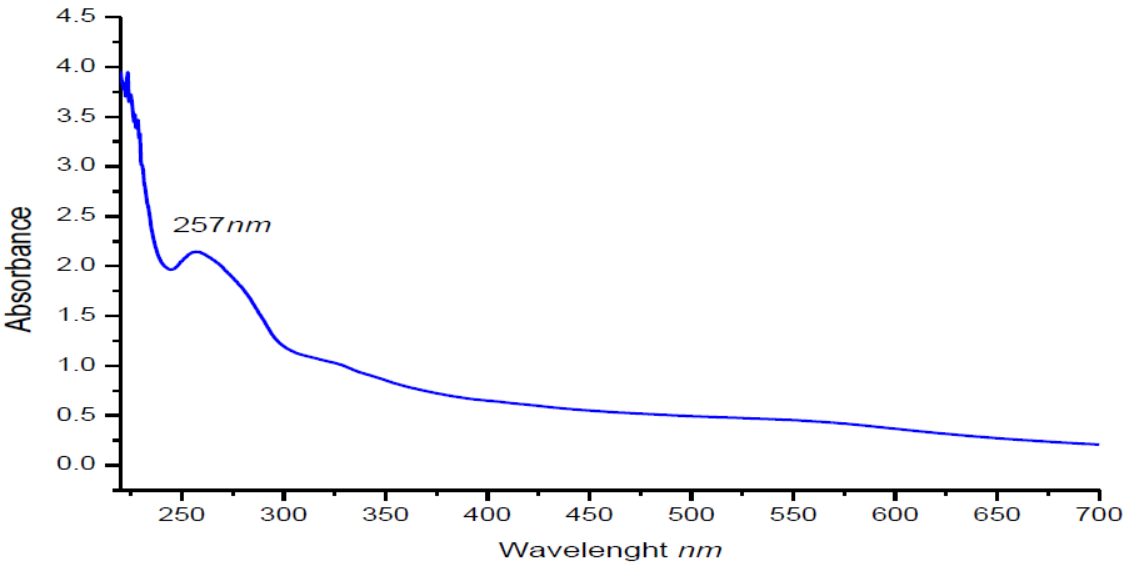

Fig. 13. UV-Visible spectroscopy graph shows the absorbance peaks of SeNPs at 257 nm.

DLS (Dynamic Light Scattering)

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 of the inorganic core along with coating material and the solvent layer attached to the particle

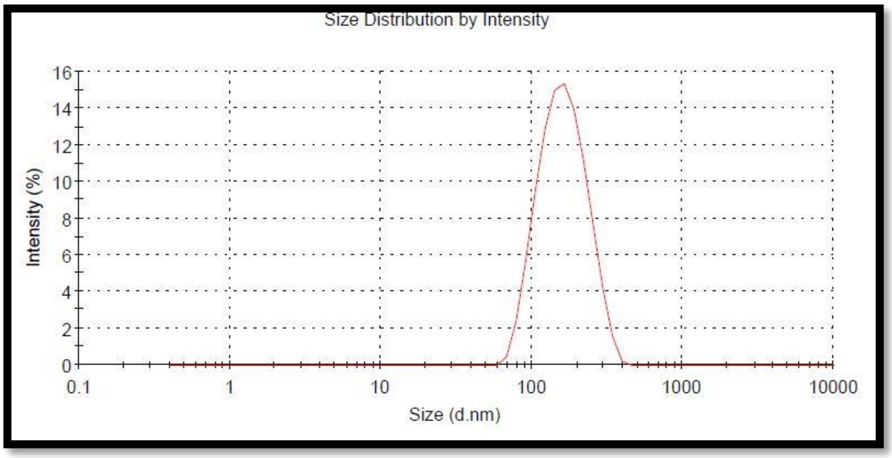

Fig. 14. Size distribution of colloidal SeNPs (Aavarage size 65 d.nm)

Zeta Potential

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

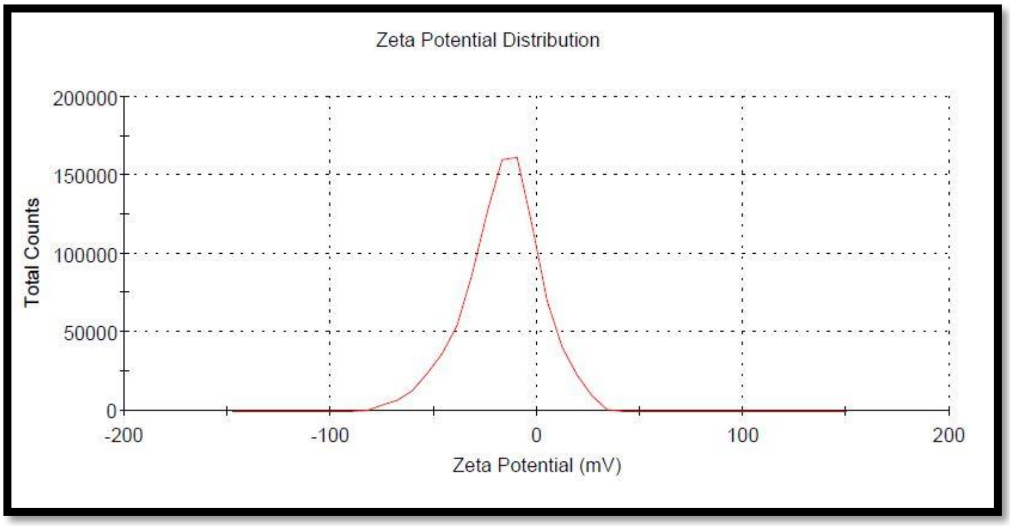

Fig. 15. Zeta potential graph SeNPs shows stability at -17mV.

Transmission electron microscopy

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

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

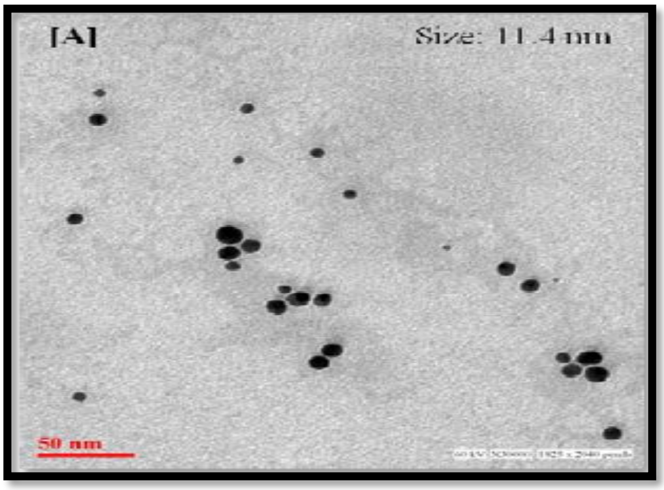

Fig 16. TEM analysis of synthesized SeNPs (26nm).

FTIR

The FTIR analysis of synthesized SeNPs 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 SeNPs. 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 confirmation. 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.

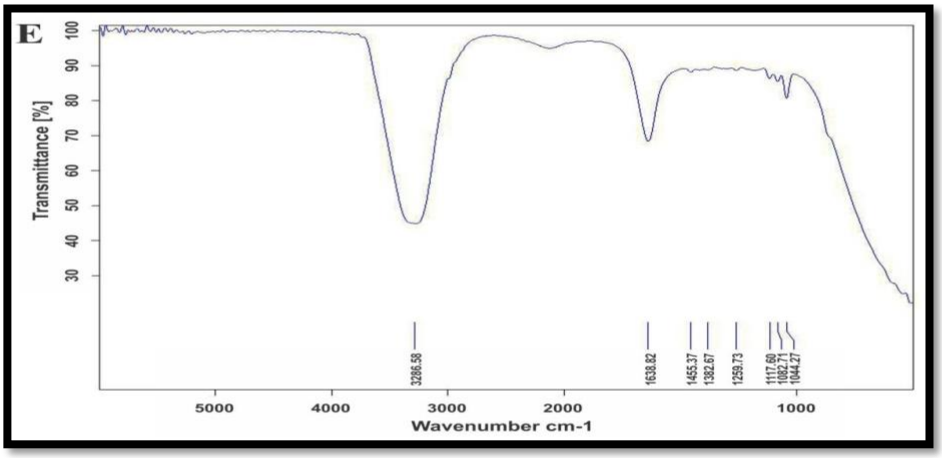

Fig. 17. FTIR analysis of Synthesized SeNPs.

Antibacterial screening

The antibacterial action of synthesized SeNPs against both gram positive and gram-negative bacterial strains was found to be satisfactory. Using the agar well diffusion method, the antibacterial potential of synthesized SeNPs was evaluated against normal and MDR strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus,* The antibacterial potential was confirmed by a clear zone of inhibition surrounding the inoculated region.

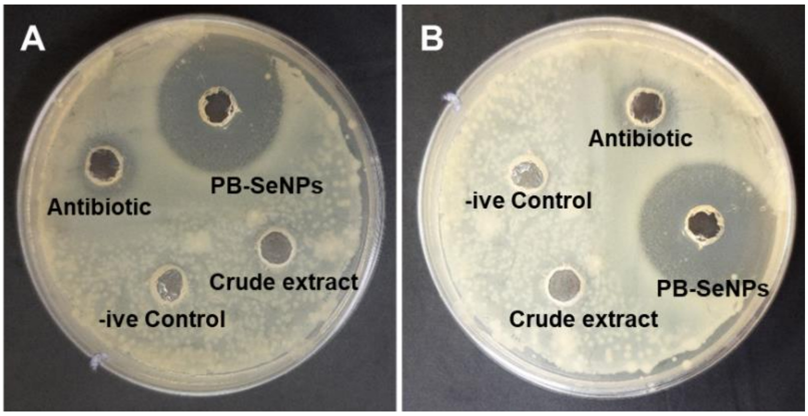

Fig. 18. Synthesized SeNPs shows Antibacterial Activity against (A) *Pseudomonas aeruginosa* and (B) *Staphylococcus aureus*.

S.No.	Bacterial strain	Zone of inhibition (mm)	
		SeNPs (50µg/ml)	**Antibiotic (levofloxacin) (50µg/ml)**
1	*Staphylococcus aureus*	**31**	**20**
2.	*Pseudomonas aeruginosa*	**30**	**25**

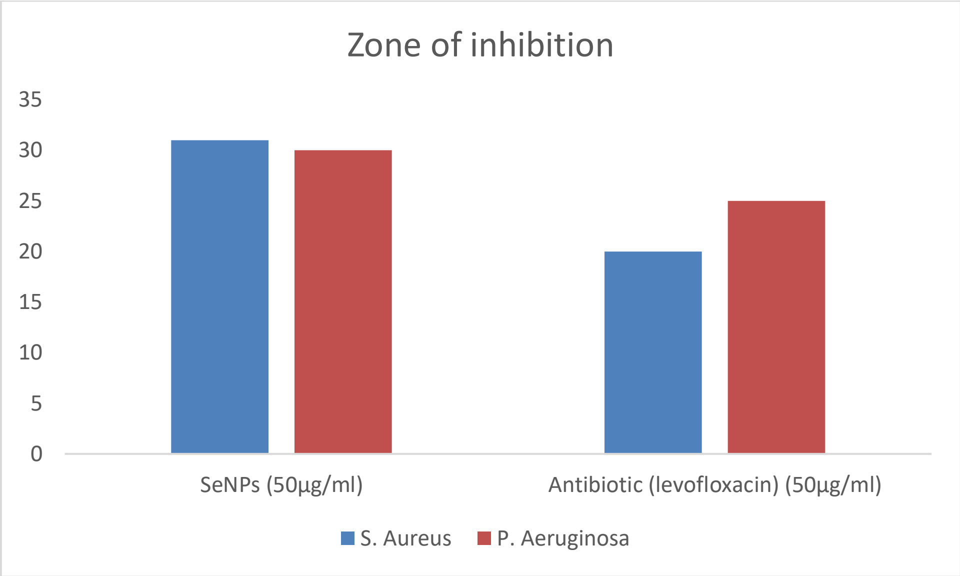

Fig. 19. The graph and table represents the zone of inhibition in mm, against above mentioned bacterial strains..

Minimal Inhibitory Concentration (MIC) Determination

The MIC is the lowest concentration of casein protein and PB-SeNPs that completely inhibits bacterial growth, and MIC_{50} is the concentration of crude extract and PB-SeNPs that inhibits 50% of the bacterial population. The MIC50 of crude extract and PB-SeNPs against Gram-negative and Gram-positive bacterial strains were recorded. However, levofloxacin was used as a standard antibiotic during the experiment. The quantified MIC50 values were *E. coli* 1.30 µg/mL and Staphylococcus aureus, 2.51 µg/ml for PB-SeNPs.

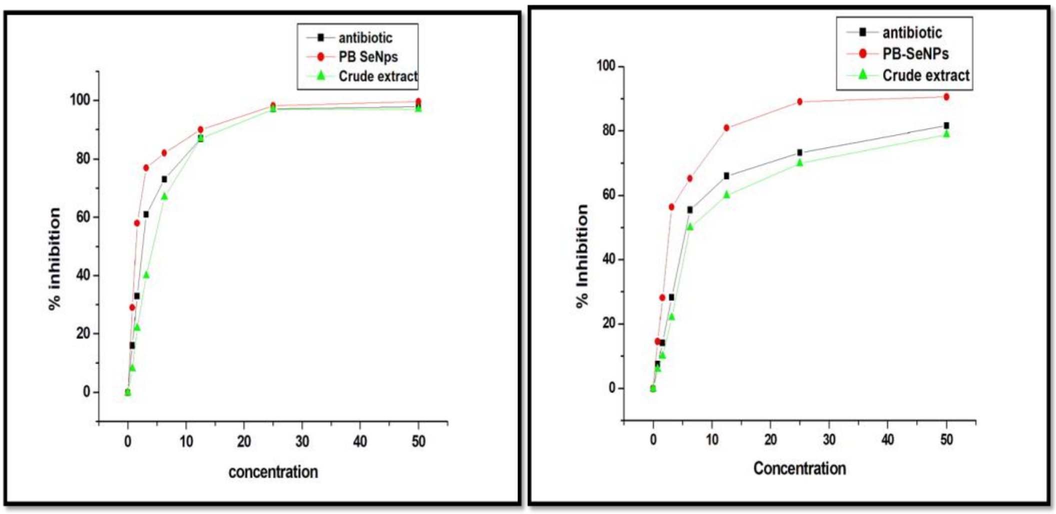

Fig. 20. IC_{50} values (8µg/ml) of leaf extract, PB-SeNPs, and Levofloxacin against (A) Pseudomonas aeruginosa, and (B) Staphylococcus aureus (96 well plate method).

Discussion and Conclusion

Discussion

PB-SeNPs were feasibly prepared by using Piper beetle leaf extract as capping and sodium borohydride as a reducing agent was used as a reducing agent. Sodium borohydride (20 mM) was used to reduce selenium powder (1 mM) at 40℃ for 3 days and leaf extract (60µl/ml) acted as a capping agent to prevent SeNPs agglomeration in dispersion, thus enhancing its stability and bioactivity under physiological conditions. Synthesized Se-NPs were characterized by UV-VIS spectroscopy which showed an absorption maximum of 257nm. The biogenic selenium nanoparticles can be utilized as potential diet supplements for selenium. However, additional studies on their bioavailability, antioxidant, antibacterial, and cytotoxic activities are envisaged. The antibacterial activity of synthesized SNPs was evaluated in vitro, we hypothesized that Se-NPs show potency against *Staphylococcus aureus and Pseudomonas strains.* The activity of Se-NPs was measured by a zone of inhibition assay, where the compounds create a circular inhibition zone, with a diameter as a read-out. The figure shows the inhibition zone results for *Staphylococcus aureus and Pseudomonas strains* respectively. Results suggested that synthesized SNPs have significant antibacterial activities.

Conclusion

Selenium is an important essential element that interferes through selenoproteins in many physiological processes of the organism and affects the production and reproductive properties. By providing an adequate supply of selenium in the diet, it is possible to effectively prevent health problems from its deficiency. Due to its high bioavailability, low toxicity, and affordability, selenium in its nano form appears to be the most appropriate for supplementation, especially in ruminants, in which traditionally used selenium compounds exhibit very low absorption in the digestive tract. The future perspective is the possibility of a global application of nanoscale selenium in nutrition as well as in clinical medicine. The development of new

Nanoparticles systems for the transport of selenium in the organism, with the possibility of modifying physicochemical properties of the particles, greater stability in the gastrointestinal tract, and allowing controlled release of selenium, offers a significant dietary and therapeutics potential. Although there are currently some concerns about the use of Se-NPs for therapeutic purposes, many scientific studies suggested that many of these generally prevailing doubts have not been confirmed. However, it is still necessary to carry out further preclinical studies in animal models.

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