A DISSERTATION ON

One-Pot Synthesis of *Citrus sinensis* Seed-Mediated Gold Nanoparticles (C-AuNPs) and Evaluation of their Antidiabetic and Antibacterial Potential

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING & INFORMATION TECHNOLOGY
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Bushra Khan**, student of **B.Tech.-M.Tech. Dual Degree Biotechnology (5th year/10th** Semester), Integral University have completed my six months dissertation work entitled **"One-Pot Synthesis of *Citrus sinensis* Seed-Mediated Gold Nanoparticles (C-AuNPs) and Evaluation of their Antidiabetic and Antibacterial Potential"** successfully from **Integral University** under the able guidance of **Dr. Zeeshan Rafi.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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This is to certify that **Ms Bushra Khan** (1800103319) has carried out the research work presented in this thesis entitled **"One-Pot Synthesis of *Citrus sinensis* Seed-Mediated Gold Nanoparticles (C-AuNPs) and Evaluation of their Antidiabetic and Antibacterial Potential"** for the award of **B.Tech.-M.Tech. Dual Degree Biotechnology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her. **B.Tech.-M.Tech. Dual Degree Biotechnology.**

I wish her good luck and bright future.

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TO WHOM IT MAY CONCERN

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I wish her good luck and bright future.

Dr. Alvina Farooqui
Professor and Head
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Bushra Khan

LIST OF ABBREVIATIONS

NPs	**Nanoparticles**
AuNPs	**Gold Nanoparticles**
C-AuNPs	***Citrus sinesis* derived Gold Nanoparticles**
SeNPs	**Selenium Nanoparticles**
TEM	**Transmission Electron Microscopy**
SEM	**Scanning Electron Microscope**
DLS	**Dynamic Light Scattering**
HIV	**Human Immunodeficiency Virus**
RSV	**Respiratory Syncytial Virus**
CVD	**Cardiovascular Disease**
NAFLD	**Non-alcoholic fatty liver disease**
SPR	**Surface Plasmon Resonance**

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1. INTRODUCTION

Nanotechnology, the field that deals with items smaller than a nanometer, has emerged as a transformative area of science and technology with the potential to revolutionize various industries. This rapidly advancing discipline encompasses the manipulation and application of nanomaterials at the nanoscale, offering unique properties and capabilities. Nanoparticles, among the key components of nanomaterials, have attracted immense attention due to their size-dependent physical and chemical properties. They are being explored extensively for a wide range of applications, from electronics and materials science to medicine and environmental remediation. The remarkable potential of nanoparticles lies in their ability to interact with matter at the atomic and molecular levels. While cells in living organisms are typically around 10 micrometers in size, the components within cells, such as proteins, can be as small as 5 nanometers (Waldchen et al., 2015). This size similarity between certain nanoparticles and cellular components has sparked interest in using nanoparticles as probes to study cellular machinery without causing significant disruption. This area of research has furthered our understanding of biological activities at the nanoscale, opening up new frontiers in nanomedicine, a branch of medicine that harnesses nanotechnology for medical applications. Nanomedicine has emerged as a transformative field, encompassing the use of nanomaterials and devices for various medical purposes. Nanotechnology has broadened the horizons of medicine, enabling advancements in disease prevention, diagnostics, targeted drug delivery, imaging, and therapy(A. U. Khan et al., 2020). The ability to engineer and manipulate nanoparticles at the molecular level allows for precision medicine and personalized treatments, leading to enhanced therapeutic outcomes and reduced side effects. Nanomedicine offers promising solutions for complex medical challenges, such as cancer treatment, neurodegenerative diseases, infectious diseases, and regenerative medicine. The rapid growth of nanotechnology and nanomedicine has spurred intensive research in the development of new nanomaterials and manufacturing techniques. Nanomaterials come in various forms, including lipid-based nanoparticles, polymeric nanoparticles, carbon-based nanoparticles, and metal-based nanoparticles. Among the metal-based nanoparticles, gold nanoparticles (AuNPs) hold a significant position in the field of nanomedicine. Their unique optical and chemical properties make them particularly attractive for a wide range of Bio-Nanotechnology applications.

One of the key advantages of gold nanoparticles is their ease of functionalization, especially through thiol connections. This property enables researchers to modify the surface of goldnanoparticles with specific ligands, antibodies, or drugs, making them highly versatile for targeted drug delivery and therapeutic applications. Additionally, gold nanoparticles have excellent biocompatibility, making them suitable for in vivo applications(Mieszawska et al., 2013). Gold nanoparticles have been extensively used as optical probes due to their distinct plasmonic properties. Their surface plasmon resonance leads to enhanced scattering and absorption of light, making them useful for various imaging and sensing applications. Researchers have also exploited their unique properties for computer-guided material production and catalysis, paving the way for the development of new materials with tailored properties.

In recent years, green synthesis methods have gained prominence for the production of nanoparticles(Heinemann et al., 2021). These approaches use environmentally friendly and cost-effective materials, such as plant extracts, to reduce metal ions and create nanoparticles. The use of plant extracts as reducing and capping agents provides a greener alternative to conventional chemical synthesis methods, reducing the use of hazardous chemicals and promoting sustainable practices.

Citrus sinensis, commonly known as sweet orange, is a widely cultivated fruit globally, known for its nutritional and medicinal properties. Sweet oranges are a rich source of vitamin C, an essential nutrient with potent antioxidant properties that strengthen the immune system and support overall health. The bioactive compounds present in citrus fruits, including flavonoids and anthocyanins, have been linked to various pharmacological effects, such as antioxidant and metal-chelating activities. Among the components of sweet oranges, the seeds have been of particular interest due to their potential bioactivity(Ni et al., 2021). In several tropical and subtropical regions, sweet orange cultivation generates a significant amount of orange seeds as waste during processing. Finding valuable uses for these seeds could enhance the value of agricultural waste and reduce its environmental impact. Studies have shown that sweet orange seeds contain bioactive chemicals, including phenolic compounds, carotenoids, tocopherols, and phytosterols(Jorge et al., 2016). These compounds exhibit potent antibacterial and anti-inflammatory properties, making sweet orange seeds a promising source for green synthesis of nanoparticles with potential therapeutic applications. Green synthesis of gold nanoparticles (C-AuNPs) using Citrus sinensis seeds as the reducing and capping agent represents a promising approach to produce nanoparticles with enhanced therapeutic potential. The one-pot synthesis method simplifies the production process and minimizes the use of harmful chemicals. In this approach, an aqueous extract of Citrus sinensis seeds is mixed with a gold precursor solution, and the bioactive compounds in the extract facilitate the reduction of gold ions to form C-AuNPs. The synthesized C-AuNPs undergo rigorous physicochemical characterization to assess their size, shape, stability, and surface functional groups.

Beyond characterizing the C-AuNPs, we investigated their potential therapeutic applications, particularly in combating bacterial infections. Antibacterial activity assessments are carried out against various bacterial strains, including both gram-positive and gram-negative strains.The antibacterial potential of C-AuNPs is evaluated using standard methods such as agar well diffusion and broth microdilution assays. The results demonstrate the promising antibacterial activity of C-AuNPs against both types of bacterial strains. The presence of bioactive chemicals from *Citrus sinensis* seeds in the nanoparticles is likely responsible for their enhanced antibacterial efficacy.

As nanotechnology and nanomedicine offer unparalleled opportunities to revolutionize various industries, especially in healthcare(Mbunge et al., 2021). The unique properties of nanoparticles, particularly gold nanoparticles, have enabled groundbreaking advancements in targeted drug delivery, imaging, and sensing. Green synthesis of nanoparticles, utilizing plant extracts as reducing and capping agents, presents a sustainable and eco-friendly alternative to traditional chemical synthesis methods(Ahmed et al., 2016). In light of the above statements, we aimed to synthesize gold nanoparticles (C-AuNPs) using *Citrus sinensis* seeds that represents a novel approach to produce nanoparticles with enhanced therapeutic potential. The natural bioactive compounds in *Citrus sinensis* seeds might serve as effective reducing and capping agents during the synthesis process, further adding to the value of this green approach. Afterwards, the antibacterial and antidiabetic potential of the C-AuNPs in comparison with standard drugs will be evaluated in the proposed study. Harnessing the capabilities of C-AuNPs may lead to innovative solutions for addressing various medical challenges and pave the way for the development of next-generation nanomedicines and nanotherapeutics. As nanotechnology continues to advance, its integration with medicine holds the promise of transforming healthcare and improving lives on a global scale.

AIM & OBJECTIVES

One-Pot Synthesis of *Citrus sinensis* Seed-Mediated Gold Nanoparticles (C-AuNPs) and evaluation of their antidiabetic and antibacterial Potential

Objectives

- One pot synthesis of Gold Nanoparticles (C-AuNPs) via *citrus sinensis* seeds
- Characterization of C-AuNPs via various Physico-chemical parameters
- Comparative antibacterial analysis of C-AuNPs in contrast to crude *citrus sinensis* seed extract and antibiotic levofloxacin.
- Antidiabetic potential analysis of C-AuNPs via alpha-amylase inhibition assay in contrast to standard inhibitor acarbose.

2. REVIEW OF LITERATURE

2.1 Introduction to nanotechnology

Nanotechnology is a multidisciplinary field that involves the design, synthesis, characterization, and utilization of materials and devices at the nanoscale level, typically measuring one billionth of a meter(Nasrollahzadeh et al., 2019). At this scale, materials exhibit unique properties due to the control over their fundamental molecular structure. The manipulation of atoms and molecules at the nanoscale allows for the creation of nanomaterials with size-dependent properties and functions(Mulvaney, 2015). This has led to significant advancements in various fields, including medicine and physiology, where materials with high specificity for subcellular interactions are essential. Nanotechnology offers the potential for precise interactions at the molecular level, enabling the development of materials and devices with high functional specificity for medical and physiological applications(R. Saini et al., 2010). The integration of traditional scientific disciplines such as chemistry, physics, biomaterials, and biology has given rise to the burgeoning field of nanotechnology, which holds promise for revolutionizing various industries.

2.2 Types of nanoparticles

Nanotechnology has revolutionized the scientific landscape by delving into the design, synthesis, characterization, and utilization of materials and devices at the nanoscale, which is one billionth of a meter. Nanomaterials, owing to their size-dependent properties, have sparked immense interest across diverse fields, offering a wide array of varieties for scientific exploration and technological applications(Shreyash et al., 2021). Among the prominent classes of nanomaterials, lipid-based nanomaterials, polymeric nanomaterials, semiconductor nanomaterials, and metallic inorganic nanoparticles stand out as crucial players in nanotechnology.

2.2.1 Lipid-Based Nanomaterials

Lipid-based nanomaterials are composed of lipids, essential biological molecules characterized by their amphiphilic nature, possessing both hydrophilic and hydrophobic properties(Zhai et al., 2019). Due to this unique feature, lipid-based nanoparticles have the ability to self-assemble into various structures, such as liposomes, micelles, and lipid nanoparticles. Liposomes are spherical vesicles with a lipid bilayer structure enclosing an aqueous core(Akbarzadeh et al., 2013). These versatile lipid-based nanomaterials have garnered significant attention in the field of drug delivery due to their biocompatibility and their capacity to encapsulate both hydrophobic and hydrophilic drugs efficiently. Lipid-based nanocarriers offer controlled and targeted drug release, facilitating enhanced drug efficacy while minimizing adverse side effects(Ahmadi et al., 2020).

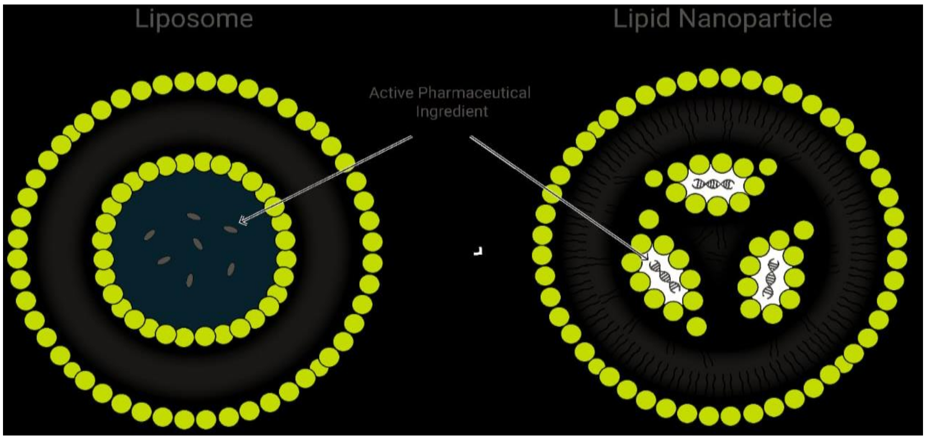

Fig.1 Liposomes and Lipid Nanoparticles as Delivery Vehicles for Personalized Medicine.

2.2.2 Polymeric Nanomaterials

Polymeric nanomaterials are comprised of synthetic or natural polymers that can be engineered into nanoparticles with diverse sizes and shapes. These nanomaterials offer exceptional tunability, biocompatibility, and biodegradability, making them ideal for a wide range of applications(Cai et al., 2021). Polymeric nanoparticles can encapsulate drugs, genes, or imaging agents, facilitating their delivery to specific target sites in the body, thereby enabling personalized and precision medicine. Furthermore, polymeric nanomaterials can be functionalized with targeting ligands, allowing selective interactions with specific cells or tissues, enhancing their specificity and therapeutic efficiency(Navya et al., 2019). The versatility of polymeric nanomaterials has led to their extensive utilization in drug delivery, cancer therapy, tissue engineering, and diagnostic imaging(Geetha Bai et al., 2018).

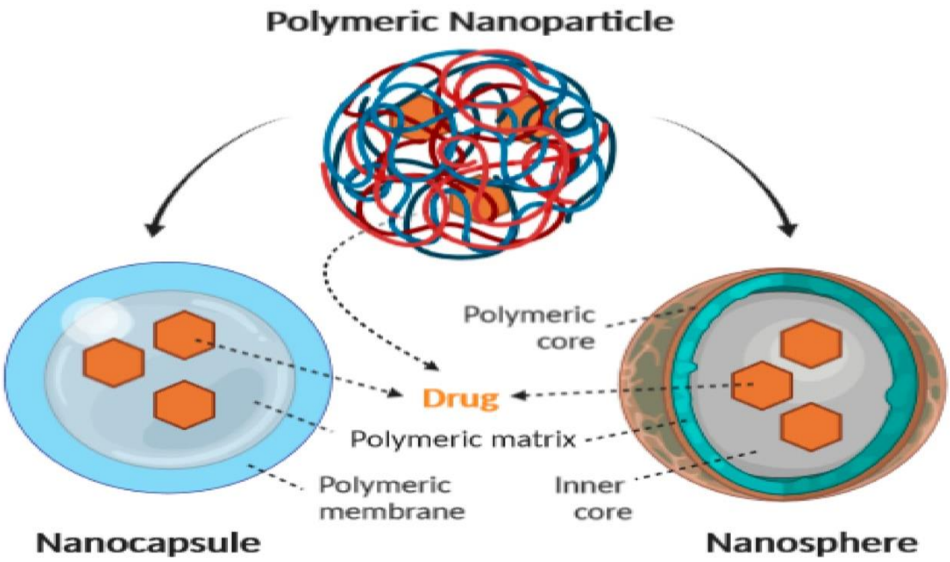

Fig.2 Polymeric Nanoparticle

2.2.3 Semiconductor Nanomaterials

Semiconductor nanoparticles exhibit both metallic and non-metallic characteristics, and their properties can be engineered by altering their size and composition(Arbuz et al., 2021). These materials find applications in electronic devices, photocatalysis, and optoelectronic devices. For instance, group II-VI semiconductor materials like ZnS, ZnO, CdS, CdSe, and CdTe have been extensively studied for their unique properties and potential applications in nanoelectronics and nanophotonics. Moreover, semiconductor nanomaterials have shown promise in biomedical imaging, as they can be functionalized with targeting moieties to enable specific cellular or tissue imaging, facilitating early disease diagnosis and targeted therapy(Han et al., 2019)

2.2.4 Metallic Inorganic Nanoparticles

Metallic nanoparticles have captivated scientists for over a century, and their usage spans across engineering and biological sciences. The ability to synthesize and modify these materials with different chemical functional groups opens up a broad spectrum of potential applications in biotechnology, magnetic separation, preconcentration of target analytes, targeted drug delivery, and diagnostic imaging. These imaging modalities require contrast agents with specific physiochemical characteristics, leading to the development of numerous nanoparticulate contrast agents, including magnetic, gold, and silver nanoparticles. Gold nanoparticles (AuNPs), for instance, exhibit unique optical and chemical properties, with their size influencing cellular uptake and targeted delivery(Her et al., 2017). On the other hand, silver nanoparticles (AuNPs) have shown potential as effective cancer treatment agents, demonstrating anticancer properties against various human cancer cells, including breast cancer cells(Vemuri et al., 2019).

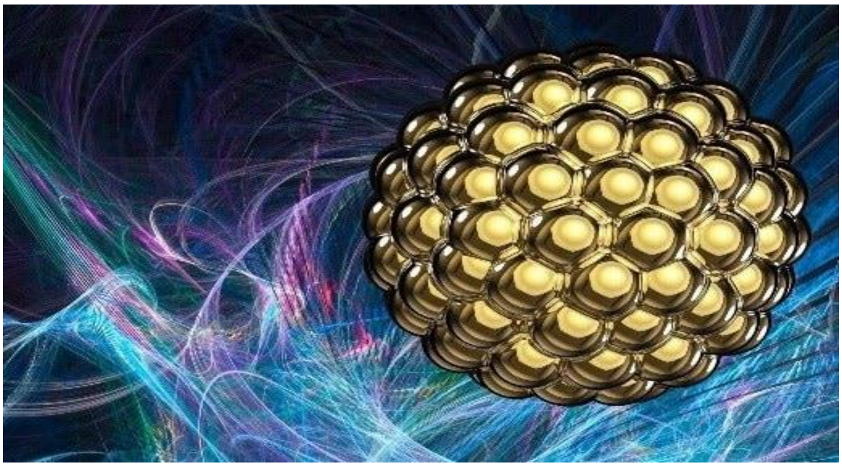

Fig.3 Metallic and Inorganic Nanoparticles

2.2.5 Iron Oxide Nanoparticles (INPs)

Iron oxide nanoparticles, such as iron (III) oxide (Fe2O3) and magnetite (Fe3O4), possess paramagnetic properties and have emerged as promising candidates for various biomedical applications. Superparamagnetic iron oxide nanoparticles (SPIONs) are of particular interest due to their ultrafine size, magnetic properties, and biocompatibility(Xie et al., 2018). These nanoparticles find applications in specific drug delivery, imaging analysis, hyperthermia, gene therapy, stem cell tracking, molecular and cellular tracking, and magnetic separation technologies, such as rapid DNA sequencing, facilitating early disease detection and improved treatment strategies.

2.2.6 Silica Nanoparticles

Silica nanoparticles, both mesoporous and nonporous, have gained significance in biological applications due to their amorphous silica structure(Jafari et al., 2019). Mesoporous silica nanoparticles, with mesopores ranging from 2 to 50 nm, find extensive use in delivering active payloads through chemical or physical adsorption. Nonporous silica nanoparticles, on the other hand, deliver through encapsulation or conjugation. These nanoparticles hold potential as enhanced drug delivery systems, allowing for the loading of drug particles and surface modification with bio-recognition entities for targeted cellular interactions. Silica nanoparticles offer the advantage of customizable inner structures for precise dispersion profiles and multifunctional applications, incorporating chemical, optical, and magnetic capabilities into a single nanostructure(Zhou et al., 2022).

2.2.7 Selenium Nanoparticles

Selenium nanoparticles (SeNPs) have garnered attention due to their high bioavailability, low toxicity, and biocompatibility, making them promising candidates for various biomedical applications(Bisht et al., 2022). Their creation can be achieved through physical, chemical, or biological techniques, with biologically synthesized SeNPs exhibiting improved compatibility with human tissues and organs. These nanoparticles hold potential for use in drug delivery, imaging, and therapeutic applications.

2.2.8 Organic Nanomaterials

Organic nanoparticles can be found in nature and form part of many industrial products. One prominent example of organic nanomaterials is dendrimers, which possess a tree-like branching configuration. Due to their unique structure, dendrimers offer precise control over drug release, making them valuable in drug delivery applications. However, the lengthy dendrimer production process remains a challenge in their application(Palan & Chatterjee, 2022).

2.2.9 Liposomes and Micelles

Liposomes, lipidic molecules resembling vacuoles, and micelles, composed of amphiphilic substances like lipids or polymers, are nanoparticles with applications in drug delivery and pharmaceutical formulations. Liposomes, ranging from 100 to 800 nm in size, possess a unilamellar structure and are known for their ability to encapsulate hydrophobic drugs(Kaur & Singh, 2023). Micelles, on the other hand, reveal their hydrophilic or hydrophobic groups depending on the surrounding media, enabling selective drug delivery to specific sites in the body(Imran & Shah, 2018).

2.3 Synthetic and assembly approaches

Synthetic and assembly approaches are fundamental to the fabrication of nanoengineered materials and devices, encompassing a wide range of experimental techniques that offer versatile capabilities(M. Yang et al., 2020). These methods involve utilizing components from solid, liquid, or gas phases to synthesize nanostructures with precise control over their properties and functionalities. In general, synthesis techniques can be categorized into two main approaches: "top-down" and "bottom-up" methods, each offering unique advantages and applications.

2.3.1 Top-Down Technique

The top-down approach involves breaking down a larger piece of material into smaller structures with specific patterns or features. One of the most well-known examples of a top-down technique is photolithography, widely employed in the semiconductor industry to fabricate integrated circuits on silicon wafers. In this process, a piece of silicon is coated with a photoresist, a photosensitive polymer. A laser is used to selectively expose the photoresist, creating patterns that will define the desired circuit elements(Lee et al., 2018). The exposed areas of silicon are then treated to function as electrodes, while the unexposed photoresist is rinsed away. Aluminium wires are subsequently deposited in the etched patterns to form transistors and other circuit components(Roberts et al., 2019).

Other nanolithographic techniques, such as dip pen nanolithography and electrostatic atomic force microscope nanolithography, enable the creation of true nanoscale features in various materials. These methods involve depositing or manipulating individual molecules into desired configurations, allowing for precise control over nanostructures.

2.3.2 Bottom-Up Technique

Contrasting the top-down approach, bottom-up techniques involve creating and synthesizing unique molecules capable of self-assembly into larger, more complex structures. The objective is to design molecules that can spontaneously organize themselves in response to specific chemical or environmental triggers, such as changes in pH, solute concentration, or the application of an electric field. Thermodynamic and molecular interactions, such as hydrophobic/hydrophilic forces, hydrogen bonds, and van der Waals interactions, play a crucial role in driving self-assembly(Y. Yang et al., 2022). Self-assembled structures can exhibit desirable chemical and physical characteristics that the individual molecules may not possess. By carefully designing the molecular interactions, researchers can create complex nanostructures with unique properties. Additionally, scaffolding or templating techniques are utilized in biological applications, leveraging pre-existing structures to direct the nucleation and growth of nanostructured materials(Huang et al., 2022).

A prominent example of bottom-up synthesis is biomineralization, where researchers develop synthetic bone biomimics. By inducing self-assembly and generating molecular small fibers interspersed with mineralized hydroxyapatite crystals, structures analogous to the collagen ultrastructure of bone can be mimicked. These techniques enable the creation of materials with properties similar to natural bone without the need for pre-existing scaffolds.

Both top-down and bottom-up approaches have their advantages and limitations, making them suitable for different applications. Top-down methods are ideal for precision fabrication of nanostructures with well-defined features, making them essential in the semiconductor industry(Persano et al., 2013). On the other hand, bottom-up approaches offer opportunities for the creation of novel materials with unique properties and functionalities, especially in biotechnology and nanomedicine(Makam & Gazit, 2018).

The synthetic and assembly approaches in nanotechnology are instrumental in developing a wide range of nanoengineered materials and devices. Through top-down methods like photolithography and bottom-up techniques involving self-assembly and templating, researchers can fabricate nanoscale structures with precise control over their properties(Diaz Fernandez et al., 2014). These advancements open up vast possibilities for various applications in electronics, medicine, and other industries, revolutionizing the future of nanotechnology.

2.4 Characterization techniques of nanoparticles

To confirm that the manufactured particles are at the nanoscale, characterization is also crucial. The term "characterization" in material science describes the general and comprehensive procedures used to investigate the structure and physical characteristics of the material. For the content to be understood scientifically, this fundamental procedure is necessary.

Some of these techniques are: -

2.4.1 Spectroscopic analysis (UV- visible spectroscopy)

The technique of UV/Visible spectroscopy is used to measure the amount of visible light that is captured and dispersed by a sample. In its most basic form, a sample is positioned between a source of light and a device called a photodetector, as well as the UV/visible light intensity is measured both before and after the sample is exposed to the light beam.

To determine the sample's wavelength dependent extinction spectrum, these measurements are contrasted at each wavelength. Usually, the data is presented as an extinction function of wavelength(David Regan et al., 2023). To make sure that spectral characteristics from the buffer itself aren't included in the sample extinction spectrum, each band is background corrected using a buffer blank.In order to identify, categorise, and research nanomaterials, UV/Vis spectroscopy is a useful tool. Silver and gold-based nanoparticles that are plasmonic have optical characteristics that are sensitive to size, shape, concentration, aggregation state, and refractive index close to the nanoparticle surface(Said et al., 2022). Using numerical models, the anticipated and measured spectra can be compared. Although non-plasmonic nanoparticles also exhibit size- and concentration-dependent optical characteristics, their spectrum is less susceptible to dispersion than that of plasmonic nanoparticles(Singh et al., 2022).

2.4.2 Transmission Electron Microscopy (TEM)

The transmission of an electron beam through a sample is imaged using the high magnification measurement technique known as transmission electron microscopy (TEM). The sample thickness—the amount of material the electron beam must pass through—and the sample material—heavier atoms scatter more electrons and have a shorter electron mean free path than lighter atoms—determine the imaging contrast through variations in the transmitted beam's amplitude and phase. Compared to light-based imaging methods, TEM imaging offers a substantially greater resolution since it illuminates the sample using electrons rather than light(Wang et al., 2023).

The contrast between the sample and the backdrop is crucial for effective nanoparticle imaging with TEM. Drying nanoparticle over a grid of copper that has a tiny layer of carbon coating is how samples are made ready for imaging. Imaging is straightforward for materials with densities of electrons substantially higher than amorphous carbon. Most metals (such as silver, gold, copper, and aluminium), most oxides (such as silica, aluminium oxide, and titanium oxide), as well as other particles, including polymeric nanoparticles, nanotubes of carbon, quantum dot particles, and magnetic nanoparticles, are included in these materials. The best technique for accurately determining the particle dimensions, grain size, distribution of size, and shape of nanoparticles is TEM imaging(Holder & Schaak, 2019). The normal margin of error for sizing is 3% of the real amount.

2.4.3 Scanning Electron Microscopy (SEM)

This is an electron microscope is a microscope that uses an electron probe—a focussed beam of electrons with comparatively low energy—that is routinely scanned over the specimen in order to directly analyse the outermost layers of solid objects. Similar to those mentioned for the transmission electron microscope (TEM), the electromagnetic lenses and electron source that create and concentrate the beam are similar. The action of the electron beam encourages the emission of low-energy secondary electrons and high-energy backscattered electrons from the specimen's surface(Kianirad et al., 2019).

Large and heavy specimens can be accommodated, and the SEM does not require complex specimen preparation procedures. In order to obtain a clear image, it is preferable to make the specimen electrically conductive. A thin layer of metal, such as gold, is often evaporated onto the specimen to produce conductivity (this thickness has no discernible impact on the precision of the surface details). However, even nonconducting specimens can be analysed without the need for a coating of metal if the SEM can be run at 1-3 kilovolts of energy(M. S. I. Khan et al., 2020).

2.4.4 Dynamic Light Scattering (DLS)

For characterising nanoparticles and other colloidal fluids, dynamic light scattering (DLS) is a crucial method(Jia et al., 2023). When a laser travels through a colloidal solution, DLS measures the light that is dispersed. The size of the particle in solution can be determined by examining the time-dependent variation of the scattered light intensity.an autocorrelation function for DLS. The function's time delay correlates to the rate of nanoparticle diffusion. The study is based on Brownian motion, which predicts that bigger particles will travel more slowly and scatter greater amounts of light than smaller particles(Tosi et al., 2020). The hydrodynamic diameter can be determined from the time dependence of the scattering intensity measurements. Because it offers details on the aggregated state of nanoparticle solutions, hydrodynamic diameter measurement is an essential complement to other size techniques like TEM. Particles in stable, unaggregated colloidal suspensions will have hydrodynamic dimensions which are similar to or slightly greater than their TEM sizes, but those in highly aggregated solutions will have hydrodynamic dimensions that are substantially bigger than the TEM size.

2.4.5 Zeta Potential

Zeta potential, commonly referred to as the electrokinetic potential, assesses the charge stability of nanoparticles with colloidal structure by measuring the "effective" charge that exists on the nanoparticle surface(Zając et al., 2023). A greater concentration of ions with the opposite charge are present close to the surface of a nanoparticle when it has a net charge on its surface, which is "screened" by the charge. The layer of ions with opposing charges moves with the nanoparticle in this layer, and this layer is often referred by the term the electrical double layer together with the layer of surface charge. The variation in potential between the main fluid in which a particle is disseminated and a layer of fluid containing the dipolar ions that is connected to the surface of the nanoparticle is measured by the Zeta Potential. When interacting with negatively charged surfaces, positive Zeta Potential particles will bond there. The Zeta Potential's magnitude gives information regarding the stability of the particles, with higher magnitude potentials displaying increased electrostatic repulsion and hence increased stability(Kay et al., 2022).

2.5 Role of green plants and its phytoconstituents in therapeutics

Plants and their phytoconstituents play a significant role in therapeutics, serving as a rich source of bioactive compounds with medicinal properties. Throughout history, traditional medicine has relied on plant-based remedies to treat various ailments, and modern medicine continues to harness the power of plant-derived compounds for therapeutic purposes(Strakhovskaya et al., 2023). Phytochemicals are secondary metabolites produced by plants, serving as quasi-nutritive compounds that aid in their defense against pathogens, insects, and environmental stressors. These phytoconstituents can also benefit humans and animals by offering protection against certain diseases caused by microbes or their toxins, owing to their antibacterial, antiviral, antifungal, and other beneficial properties.

2.5.1 Therapeutic Properties

2.5.1.1 Antibacterial Properties: Antibacterial phytoconstituents have emerged as promising alternatives to traditional antibiotic treatments, particularly in combating infections caused by *Helicobacter pylori*, a bacterium responsible for peptic ulcers and distal stomach cancer(Saxena et al., 2020). While conventional antibiotics have been effective in treating such infections, the rise of antibiotic resistance poses a significant challenge to modern medicine. Phytochemicals derived from plants, such as alkaloids, polysaccharides, and flavonoids, offer a potential solution to this problem. Alkaloids, a class of natural compounds commonly found in various plants, have shown significant antibacterial activity against *H. pylori*(Reddy et al., 2023). Studies have highlighted the efficacy of specific alkaloids, such as *berberine* from *Berberis* species, against this bacterium(Malhotra et al., 2021). *Berberine* inhibits *H. pylori* growth and adherence to gastric mucosa, reducing the risk of peptic ulcers.

Polysaccharides, another group of phytochemicals, have also exhibited antibacterial properties against *H. pylori*. These complex carbohydrate molecules, found in plants like Aloe vera and Echinacea, can interfere with bacterial adhesion and disrupt biofilm formation, hindering the bacterium's ability to establish infection. Flavonoids, a diverse group of polyphenolic compounds abundant in fruits, vegetables, and medicinal plants, have demonstrated potent antibacterial effects against *H. pylori*(Mueed et al., 2023). Quercetin, a flavonoid present in apples, onions, and green tea, has been shown to inhibit *H. pylori* growth and reduce its virulence.

Moreover, recent research has uncovered the potential of carrot seed oil as an effective in vitro treatment for *H. pylori* infections(Rakha et al., 2023). The oil extracted from carrot seeds contains bioactive compounds with antibacterial properties, presenting a promising avenue for developing novel therapeutic agents against this bacterium.

2.5.1.2 Antiviral Properties: Viral infections pose unique challenges in medical treatment, as viruses are highly adaptable and can rapidly develop resistance to antiviral medications. Natural products, including plant extracts and pure compounds, have gained attention for their potential as antiviral agents. Phytochemicals derived from plants offer a diverse array of mechanisms to combat viral infections and hold promise in developing new antiviral medications(Jantan et al., 2023). In the search for effective antiviral agents, scientists have explored plant extracts and their bioactive components(Biswas et al., 2020). These compounds can target various stages of the viral life cycle, inhibiting viral replication, entry into host cells, and assembly of new virions. Some plant-derived compounds, such as polyphenols found in green tea and resveratrol in grapes, have demonstrated potent antiviral properties against a range of viruses, including influenza, herpes simplex virus, and human immunodeficiency virus (HIV). These polyphenols interfere with viral attachment to host cells and viral enzyme activities essential for replication. Additionally, essential oils extracted from certain aromatic plants have shown antiviral effects(Mustafa et al., 2023). Tea tree oil, derived from the leaves of *Melaleuca alternifolia*, has exhibited antiviral activity against herpes simplex virus and influenza virus(Iacovelli et al., 2023). Eucalyptus oil has also demonstrated inhibitory effects on respiratory syncytial virus (RSV), a common cause of respiratory infections. Naturally occurring flavonoids, such as quercetin and catechin, have been studied extensively for their antiviral potential. Quercetin, in addition to its antibacterial properties, has shown efficacy against various viruses, including HIV, hepatitis B and C viruses, and respiratory viruses(Di Petrillo et al., 2022). These plant-derived compounds show promise as potential candidates for the development of novel antiviral medications, offering hope in the fight against viral infections and addressing the challenges posed by viral resistance to conventional drugs.

2.5.1.3 Antifungal Properties: Fungal infections, often resistant to conventional antifungal agents, have become a growing concern in healthcare settings. Phytochemicals with antifungal properties offer an alternative approach to combat these infections, with the potential to overcome drug resistance and reduce side effects(Murugaiyan et al., 2022).Phytochemicals exhibit antifungal properties through various mechanisms, targeting fungal cell structures and functions to disrupt their growth and survival. One such mechanism involves interfering with cell membrane permeability, causing the leakage of essential cellular components and ultimately leading to fungal cell death.

Compounds like saponins, found in plants like soapwort (*Saponaria officinalis*), possess antifungal properties by disrupting cell membrane integrity. These saponins have shown efficacy against various pathogenic fungi, including Candida species and *Aspergillus fumigatus.* Phytochemicals can also alter redox and osmotic balance within fungal cells, creating unfavourable conditions for their survival. For example, polyphenols such as tannins and flavonoids, found in plants like tea, grapes, and pomegranates, can induce oxidative stress in fungal cells, leading to their destruction(Wu et al., 2020).Inhibition of key enzymes involved in fungal cell wall formation, mitochondria, and cytoplasm can also disrupt fungal growth. Compounds like terpenoids, abundant in essential oils derived from plants like thyme and oregano, have shown potent antifungal activity by targeting these essential cellular processes.

Furthermore, some herbal plants have demonstrated antifungal effects against specific phytotoxic fungal strains(Xu et al., 2021). The application of these plants as biofungicides could offer a natural and eco-friendly solution to prevent food spoilage and reduce reliance on synthetic fungicides.

2.5.2 Metabolic Disorders:

2.5.2.1 Obesity: Obesity has become a global epidemic and is associated with a range of comorbidities that significantly impact public health. The prevalence of obesity-related conditions, such as type 2 diabetes, cardiovascular disease, and certain cancers, highlights the urgency to explore alternative treatment options. Plant-based therapies, when combined with lifestyle modifications, can play a crucial role in managing and preventing obesity-related complications. Adopting a healthy lifestyle that includes regular physical activity, a balanced diet, and reduced consumption of saturated fats, sugar, and salt is essential in managing obesity. Additionally, incorporating plant-based foods rich in fiber, vitamins, and antioxidants can aid in weight management(*Top 20 High-Fiber Foods and Why You Should Eat*, n.d.). Fruits, vegetables, whole grains, legumes, and nuts are excellent choices for promoting satiety and supporting weight loss efforts.Medicinal plants have been traditionally used to manage weight-related issues and metabolic disorders. Active compounds found in these plants can help regulate appetite, enhance metabolism, and modulate fat metabolism. For example, certain herbal extracts containing bioactive compounds, such as catechins found in green tea, have been shown to increase thermogenesis and fat oxidation, contributing to weight loss.

Moreover, phytochemicals with anti-inflammatory properties found in turmeric, ginger, and garlic can help reduce chronic inflammation often associated with obesity and its related conditions(Adetuyi et al., 2023). By addressing inflammation, these plant-based remedies may mitigate the risk of developing insulin resistance and cardiovascular complications.

2.5.2.2 Diabetes Mellitus: The rising prevalence of diabetes mellitus has become a significant health concern globally. Management of diabetes revolves around maintaining blood glucose levels within a healthy range. Medicinal plants have shown promising potential in this regard, as they contain bioactive compounds that can positively impact glucose metabolism.Certain medicinal plants, such as *Cymbopogon citratus* (lemongrass) and *Cucurbita pepo* (pumpkin), contain -glucosidase and -amylase inhibitors(Nadeeshani Dilhara Gamage et al., 2022). These inhibitors can delay the breakdown of carbohydrates in the digestive tract, leading to a slower and more controlled rise in post-meal blood glucose levels. As a result, incorporating these plant-derived compounds into diabetic management can help stabilize blood sugar levels and reduce post-prandial hyperglycaemia. Moreover, studies have indicated that medicinal plants like *Gymnema sylvestre* can enhance the uptake of glucose in cultured cells, including hepatic cells, muscle cells, and preadipocytes(Rashmi et al., 2020). This increased glucose uptake can lead to better glycemic control, which is particularly beneficial for individuals with type 2 diabetes.

2.5.2.3 Cardiovascular disease (CVD): Cardiovascular diseases, including atherosclerosis, hypertension, congestive heart failure, and ischemic heart disease, are leading causes of morbidity and mortality worldwide. Plant-derived compounds have been investigated for their potential in preventing and managing various CVDs. Certain plant extracts have shown the ability to lower blood pressure and reduce the risk of hypertension, a significant risk factor for CVD. Herbal remedies like garlic extract and hawthorn have demonstrated vasodilatory effects, leading to improved blood flow and decreased blood pressure.

Phytochemicals, such as flavonoids found in berries and citrus fruits, possess antioxidant and anti-inflammatory properties that can protect blood vessels and prevent the development of atherosclerosis(Santa et al., 2023). By reducing oxidative stress and inflammation, these compounds may lower the risk of plaque formation and subsequent cardiovascular complications.

Additionally, plant-based diets rich in fruits, vegetables, and whole grains can contribute to better cardiovascular health by reducing cholesterol levels and promoting overall cardiovascular well-being(Kalpe et al., 2023). Including these foods in the diet can help manage CVD risk factors and improve heart health.

2.5.2.4 Non-Alcoholic Fatty Liver Disease: Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of excess fat in the liver, leading to inflammation and impaired liver function(Cheon & Song, 2022). Plant-based treatments have shown promise in mitigating the effects of NAFLD and improving liver health. Certain plant extracts contain bioactive compounds with hepatoprotective properties, such as silymarin from milk thistle and curcumin from turmeric. These compounds can help reduce liver inflammation and oxidative stress, leading to improved liver function and a reduction in fat accumulation.

Moreover, incorporating a plant-based diet can support liver health by providing essential nutrients and antioxidants that protect liver cells from damage. Fruits, vegetables, and herbs like artichoke and dandelion have been traditionally used for their liver-cleansing properties, making them valuable additions to NAFLD management strategies.

2.5.2.5 Cancer: Cancer chemoprevention, which involves the use of agents to inhibit the early stages of carcinogenesis or prevent the invasion of premalignant cells, has gained attention as a potential strategy to reduce cancer incidence. Phytochemicals derived from medicinal plants hold promise as chemopreventive agents, offering a natural and potentially effective approach to cancer prevention(Islam et al., 2022).

Numerous phytochemicals have demonstrated anticancer properties, including flavonoids, polyphenols, and terpenoids. These compounds can target various pathways involved in cancer development, such as inhibiting cancer cell proliferation, inducing apoptosis (programmed cell death), and suppressing tumor angiogenesis (formation of new blood vessels to support tumor growth).

Furthermore, certain plant-based diets, such as the Mediterranean diet and traditional Asian diets rich in fruits, vegetables, whole grains, and herbs, have been associated with a reduced risk of certain cancers(Tsai et al., 2023). These diets are known for their high content of phytochemicals and antioxidants, which play a role in protecting cells from damage and reducing cancer risk.

Green plants and their phytoconstituents offer a treasure trove of bioactive compounds with therapeutic potential. From antimicrobial properties, combating bacterial, viral, and fungal infections, to their role in managing metabolic disorders like obesity and diabetes, plant-derived compounds are invaluable in modern medicine. Their ability to prevent cardiovascular diseases and contribute to cancer chemoprevention further highlights their significance in therapeutics. As researchers continue to explore the diverse array of phytochemicals, the integration of plant-based treatments into mainstream medicine is likely to expand, offering new and effective therapeutic options for various health conditions. Embracing the ancient wisdom of traditional medicine and combining it with cutting-edge scientific research paves the way for a more holistic and effective approach to healthcare.

2.6 Role of orange, its seeds and other components in medical field

Orange, commonly known as santra or naranga, is a popular fruit appreciated for its delightful taste and high vitamin C content. Beyond its well-known nutritional benefits, oranges and their various components offer a treasure trove of bioactive compounds with therapeutic potential, making them valuable in modern medicine.

2.6.1 Potential Uses of Oranges for Heart Health: Citrus fruits, including oranges, have been associated with potential benefits for heart health. They contain compounds like vitamin C, flavonoids, and carotenoids, which may have protective effects on the heart. Additionally, orange peels contain polymethoxylated flavones (PMFs) that have been studied for their cholesterol-lowering properties(R. K. Saini et al., 2022). While more research is needed to fully understand the impact on human heart health, incorporating oranges and their peel into the diet may contribute to cardiovascular well-being.

2.6.2 Potential Uses of Oranges for Cancer Prevention: Oranges are rich in limonene, a compound believed to have anti-cancer properties. Limonene has been associated with a reduced risk of various cancers, including those of the stomach, skin, lung, and breast. The antioxidant activity of oranges may also play a role in their potential anti-cancer effects(Caponio et al., 2022). However, it is essential to consult with a healthcare professional before using oranges or any fruit as a substitute for medical treatment in cancer cases.

2.6.3 Potential Uses of Oranges for Ulcer Prevention: Studies suggest that regular consumption of orange juice may lower the risk of contracting *Helicobacter pylori* infection, which is responsible for stomach ulcers(Elbehiry et al., 2023). The presence of fresh orange juice may have some impact on ulcer development. However, it is crucial to follow medical advice and avoid using any fruit or plant as a treatment without consulting a healthcare professional(Caprara et al., 2021).

2.6.4 Potential Uses of Oranges for Anxiety Relief: Sweet orange oil, derived from oranges, is believed to have anxiolytic properties and is used in aromatherapy to reduce anxiety and tension. While aromatherapy can be beneficial for some individuals, those experiencing anxiety should seek guidance from a physician or psychiatrist before using orange oil(Hedigan et al., 2023).

2.6.5 Potential Uses of Oranges for Diabetes Management: Orange peels contain bioflavonoids, which have been studied for their blood glucose-lowering properties(Shehadeh et al., 2021). These compounds may control enzymes that regulate glucose levels, making them potentially beneficial for individuals with diabetes. However, it is crucial to follow medical recommendations and prescriptions for diabetes management and consult a doctor before using oranges or any fruit to treat diabetes.

2.6.6Other Potential Uses of Oranges: Oranges offer a host of health benefits due to their high vitamin C content. Vitamin C supports the immune system, helping to protect against frequent colds, coughs, and ear infections. Additionally, orange juice without added sugar and salt may help alleviate symptoms of inflammatory disorders like osteoarthritis, rheumatoid arthritis, and asthma(Quispe et al., 2021). Studies have indicated that regular consumption of orange juice increases citric acid excretion and urine pH values, reducing the risk of kidney stone formation(Barghouthy & Somani, 2021). Furthermore, orange poultices made from roasted orange pulp can be used to treat certain skin conditions, while dried orange flowers and leaves have been employed in France and Italy as heart-protective, antiemetic, and antispasmodic remedies.

While research shows the potential advantages of oranges in various contexts, it is crucial to consult with a physician or qualified healthcare professional before using oranges or any other natural remedy to treat medical conditions. Additional research is needed to fully comprehend the broad range of health benefits that oranges may provide to individuals.

2.7 Role of nanotechnology in increasing the efficacy of herbal compounds:

Ancient traditional medicine has long embraced herbal treatments for various ailments(Tugume & Nyakoojo, 2019). Advances in phytochemical and phytopharmacological studies have allowed us to understand the chemical makeup and biological properties of numerous therapeutic plant products. However, the efficacy of many therapeutic plant species is limited by the availability and bioavailability of their active ingredients(Johnson et al., 2021). Many biologically active components in herbal extracts, such as flavonoids, tannins, and terpenoids, are highly soluble in water but poorly absorbed due to factors like their inability to pass through cell membranes, large molecular size, or poor absorption rates. These limitations reduce the bioavailability and effectiveness of certain extracts, limiting their clinical use. To overcome these barriers, researchers have suggested combining herbal medicine with nanotechnology. Nanostructured systems offer the potential to enhance the action of plant extracts by increasing their bioavailability, reducing required doses, minimizing adverse effects, and optimizing their activity throughout the treatment period.

By utilizing nano systems, active herbal ingredients can be effectively targeted to the intended site of action, ensuring sustained delivery at therapeutic concentrations(Safta et al., 2022). This level of precision and control is challenging to achieve with conventional therapies. Integrating nanotechnology into herbal medicine can potentially revolutionize traditional treatments, offering more efficient and effective therapeutic options for various health conditions. Nanotechnology-based approaches, such as nanoencapsulation and nanoparticle delivery systems, have shown promising results in improving the bioavailability of herbal compounds(Rudrapal et al., 2022). These advancements hold the potential to optimize the therapeutic benefits of herbal remedies, making them more reliable and predictable in their effects.

The investigation of synthesizing gold nanoparticles from *Citrus sinensis* (orange) seeds was chosen due to the growing interest in nanotechnology and its potential applications in medicine and healthcare. Gold nanoparticles have shown promising properties, such as excellent biocompatibility, tunable size, and unique optical properties, making them attractive for various biomedical applications, including drug delivery, imaging, and cancer therapy.

Citrus sinensis seeds are readily available, and they contain various phytoconstituents, including flavonoids, phenolics, and terpenoids, which are known for their antioxidant and bioactive properties. By using these seeds as a natural source for the synthesis of gold nanoparticles, we aimed to explore a green and sustainable approach to produce nanoparticles without relying on harmful chemicals or expensive equipment.

With the proposed study, we aimed to lay the foundation for further research and potential commercialization of gold nanoparticles synthesized from *Citrus sinensis* seeds as a safe, effective, and environmentally friendly nanomaterial for various biomedical applications. Moreover, the study aimed to highlight the importance of exploring natural sources and green synthesis methods in nanotechnology to advance the field while minimizing the environmental impact.

3.MATERIAL AND METHODS

3.1 *Citrus sinensis* seed collection and aqueous extract preparation

Seeds of *Citrus sinensis* fruit were procured from the local market in Lucknow and subjected to a thorough washing process using distilled water to eliminate surface impurities. Subsequently, the seeds were air-dried and finely powdered. An aqueous extract was prepared by mixing 50 grams of the dried seed powder with 100 mL of double-distilled water. The resulting solution was then concentrated to obtain a stock concentration (C-Aq) of 500 mg/mL, which was stored at 4°C for future experimental use.

3.2 *Citrus sinensis* seed extract derived gold nanoparticles (C-AuNPs)

For the synthesis of gold nanoparticles (C-AuNPs), a 1 mL reaction mixture was prepared, containing 1 mM Chloroauric acid (HAuCl4) and a phosphate buffer (50 mM, pH 7.4), supplemented with 60 µL of C-Aq (aqueous extract of Citrus sinensis seeds). The reaction mixture was then incubated for 48 hours to observe any initial color changes indicative of nanoparticle formation. A standard C-Aq extract was used as a control.

After the 48-hour incubation period, the samples were analyzed using a UV-Visible spectrophotometer (Eppendorf) with a cuvette of path length 1 cm to validate the successful synthesis of C-AuNPs. Subsequently, the samples were filtered using a syringe filter with a pore size of 2 µm to remove any unbound constituents or proteins. A 50% v/v ethanol treatment was applied to further eliminate impurities. The samples were then subjected to centrifugation at 30,000× g for 30 minutes. Finally, the samples were washed twice with Milli Q water to ensure the purity of the synthesized C-AuNPs.

3.3 Characterization of C-AuNPs

3.3.1 UV- Vis spectroscopy

To investigate the successful synthesis of C-AuNPs, UV-Visible spectrophotometry was employed. After 48 hours of incubation, the UV-Spectrum of the reaction samples was recorded, covering a wavelength range of 200-800 nm. This technique is widely used for the characterization of nanoparticles because it allows us to monitor the changes in absorbance associated with the formation of nanoparticles(Irshad et al., 2021).

The basis of UV-Visible spectrophotometry for nanoparticle synthesis lies in the phenomenon of "Surface Plasmon Resonance" (SPR)(Saeed et al., 2020). When metal salts, such as Chloroauric acid (HAuCl4) used in this study, are reduced in the presence of the Citrus sinensis seed extract, they undergo a transformation into gold nanoparticles. As a result of this reduction, the electrons in the nanoparticles oscillate collectively, creating a resonance effect, which leads to the absorption of specific wavelengths of light. This absorbed light causes a distinctive change in the color of the reaction mixture, which can be observed visually as well as quantitatively using the spectrophotometer.

The characteristic color change from the initial color of the metal salt solution to the appearance of a distinct color, often varying from red to purple depending on the particle size, indicates the successful formation of C-AuNPs. The peak absorbance wavelength in the UV-Visible spectrum corresponds to the Surface Plasmon Resonance, and its intensity correlates with the concentration and size of the nanoparticles. The higher the intensity of the peak, the greater the number of nanoparticles formed.

3.3.2 Transmission Electron Microscopy (TEM):

For performing TEM analysis to determine the size and shape of the nanoparticles, a single drop of an AuNPs suspension was applied to carbon-coated copper TEM grids. It was then analyzed utilizing a a Tecnai G2 Spirit transmission electron microscope equipped with a BioTwin lens configuration. An 80 kV80-kV accelerating voltage powers the system.

3.3.3 DLS and Zeta potential for size and stability determination:

To assess the size and stability of the synthesized nanoparticles, Dynamic Light Scattering (DLS) and Zeta potential measurements were performed. DLS is a powerful technique that measures the hydrodynamic radius of nanoparticles in solution, providing valuable information about their size distribution. For DLS analysis, a dynamic light scattering particle size analyzer (Zeta Sizer Nano-ZS, Model ZEN3600, Malvern Instrument Ltd, Malvern, UK) was utilized. A sample of the conjugated C-AuNPs was taken in a DTS0112-low volume disposable sizing cuvette of 1.5 ml. To ensure accurate measurements, the sample powder was first diluted to a concentration of 0.5% (w/v) in deionized water.

Prior to DLS analysis, the particles were subjected to sonication using Sonic & Material Inc., New Town, CT, USA, at 30 watts for 1 minute (20 seconds on and 5 seconds off). This step aimed to promote uniform dispersion of the nanoparticles in the solution.

The mean particle size was determined as the average of triplicate measurements for a single sample, ensuring reliable and reproducible results. Furthermore, the surface charge of the C-AuNPs was assessed using the Zeta Sizer Nano-ZS, Model ZEN3600 (Malvern Instrument Ltd, Malvern, UK). Zeta potential is a critical parameter that provides insights into the stability and potential interactions of nanoparticles in a colloidal solution.

3.4 Antibacterial potential analysis of C-AuNPs against gram positive and gram negative bacterial strains in contrast to standard antibiotic levofloxacin

3.4.1 Determination of zone of inhibition

The antibacterial efficacy of C-Aq and C-AuNPs was evaluated using the disc diffusion method. The pure cultures of *Gram-negative Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 15692)* were acquired from the American Type Culture Collection for the antibacterial test study. The Microbial Type Culture Collection and Gene Bank (MTCC), located at the Institute of Microbial Technology (IMTECH), Chandigarh, India, is where researchers were able to find the Gram-positive *Bascillus subtilis (MTCC 8114).* The studied strains' suspensions were calibrated to 0.5 McFarland. In order to conduct the study, the suspension was applied to the surface of Mueller-Hinton (M.H.) agar after being obtained from nighttime trypticase soy broth (TSB) cultures. The wells of MH agar plates were filled with 50µLof various concentrations of C-AuNPs (10, 20, 40, 60, and 80 mg/mL), as well as various concentrations of crude C-Aq (10, 25, 50, 75, and 100 mg/mL), PBS as a negative control, and amoxicillin (25 mg/mL) as a positive control, during the experiment. The plates of agar were subjected to incubation overnight at 37 °C during the triplicate experiments. The inhibitory zone's diameter was subsequently measured. Under the identical experimental settings, the experiment was carried out three times.

3.4.2 Minimal inhibitory concentration determination

Using the disc diffusion method, the antibacterial efficacy of C-Aq and C-AuNPs was assessed. As previously mentioned, the bacterial suspensions were applied to the surface of Mueller-Hinton (M.H.) agar after being standardised to 0.5 McFarland and acquired from overnight cultures in trypticase soy broth (TSB). During the experiment, 50 µL of different concentrations of C-AuNPs (10, 20, 40, 60, and 80 mg/mL), various concentrations of crude C-Aq (10, 25, 50, 75, and 100 mg/mL), a negative control (PBS), and a positive control amoxicillin (25 mg/mL) were added to the wells of MH agar plates. The measurement of the circumference of the area of inhibition was determined after the agar plates had been incubated overnight at 37°C. The experiment was carried out in duplicate under the same circumstances.

3.5 Alpha Amylase Enzyme Inhibition Assay for Antidiabetic Potential:

To evaluate the antidiabetic potential of C-AuNPs, an alpha amylase enzyme inhibition assay was performed. The assay measures the ability of C-AuNPs to inhibit the activity of the alpha amylase enzyme, which plays a key role in carbohydrate digestion. The assay was carried out by incubating C-AuNPs with alpha amylase enzyme and a starch substrate. The enzyme catalyzes the hydrolysis of starch into simpler carbohydrates, resulting in the formation of reducing sugars. However, in the presence of C-AuNPs, the enzyme's activity is inhibited, leading to a decrease in the formation of reducing sugars. The extent of inhibition was measured spectrophotometrically, and the percentage of alpha amylase inhibition was calculated. The assay provides valuable insights into the potential of C-AuNPs as a natural antidiabetic agent by inhibiting the breakdown of complex carbohydrates into glucose, thereby aiding in the management of blood glucose levels. The experiment was conducted in triplicate to ensure the accuracy and reliability of the results.

4. RESULTS AND DISCUSSION

4.1 Characterization of synthesised C-AuNPs

4.1.1 UV Visible Spectroscopy

The successful synthesis of gold nanoparticles (C-AuNPs) was achieved, and the confirmation of their formation was obtained through UV-Visible spectroscopy. Initially, the reaction mixture exhibited a distinctive ruby red color, which served as a visual indication of the successful synthesis of AuNPs (fig.4). Subsequently, the UV-Vis spectrum of the synthesized AuNPs displayed a characteristic peak absorbance at 530 nm (fig.5). This prominent peak is attributed to the Surface Plasmon Resonance (SPR) phenomena exhibited by the gold nanoparticles. The SPR phenomenon arises from the collective oscillation of conduction band electrons in the AuNPs, leading to the absorption of light at specific wavelengths, resulting in the observed peak in the UV-Vis spectrum.

The presence of the SPR peak at 530 nm is a clear indication of the successful formation of gold nanoparticles with distinct optical properties. The unique optical characteristics exhibited by AuNPs make them highly valuable for a wide range of applications, including in biomedicine, sensing, and catalysis. The ruby red color and the SPR peak in the UV-Vis spectrum provide strong evidence of the successful synthesis of C-AuNPs.

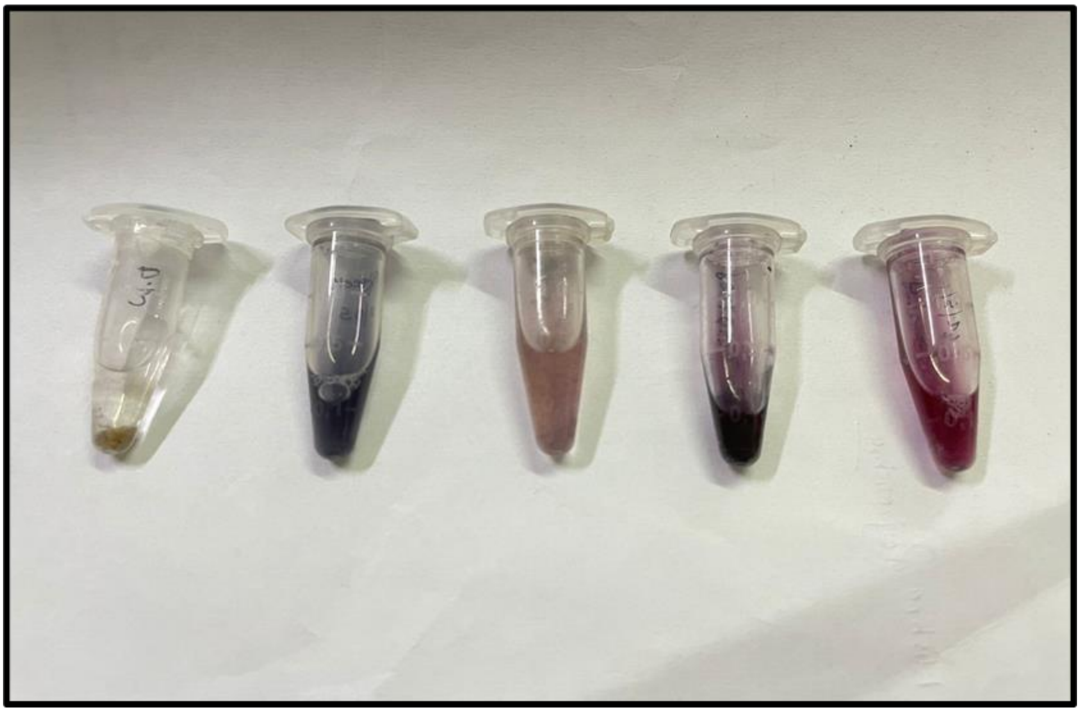

Fig.4 Successful synthesis of C-AuNPs (Ruby red colour)

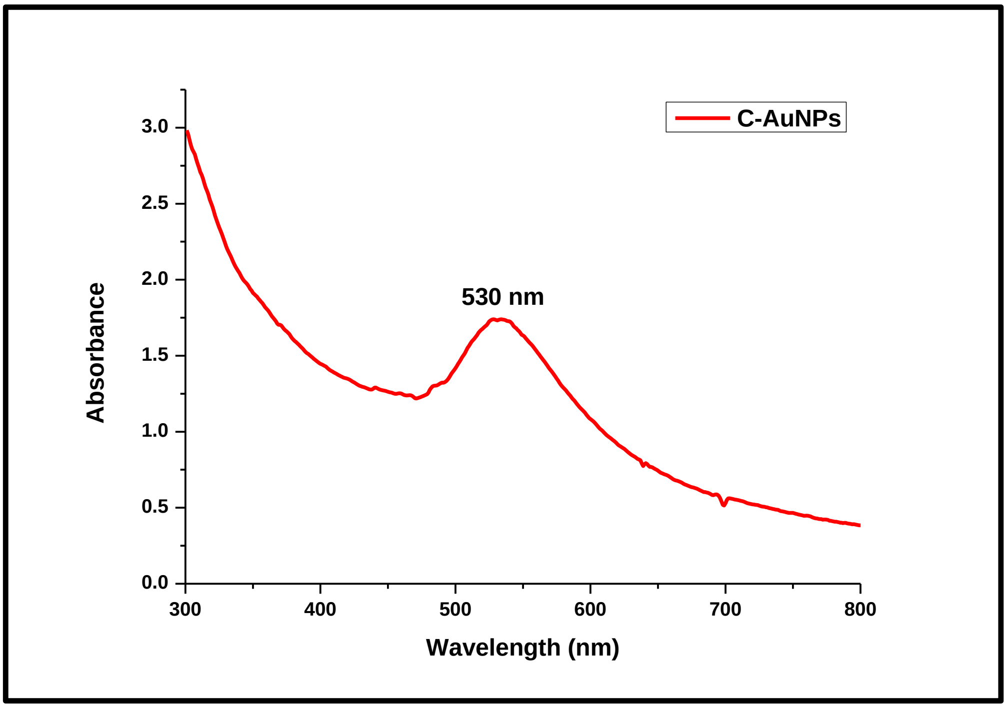

Fig.5 UV-Visible spectra of C-AuNPs.

4.1.2 Transmission Electron Microscope (TEM)

TEM analysis provided high-resolution micrographs of the AuNPs, allowing for the examination of individual nanoparticles in detail. The micrographs revealed well-defined and distinct spherical shapes, indicating the successful synthesis of uniformly shaped nanoparticles (fig.6). Particle size is a critical parameter that influences the optical, catalytic, and biological properties of nanoparticles. The TEM micrographs indicated an average particle size of approximately 22 nm for the synthesized AuNPs. The relatively small and uniform size of the nanoparticles makes them highly desirable for biomedical applications, where the efficient delivery of drugs or targeting specific cellular structures requires nanoparticles of precise dimensions. Moreover, the absence of agglomeration or aggregation in the TEM micrographs further reinforces the efficiency of the 22 nm stabilizing or capping agent used during the synthesis process. Agglomeration or aggregation of nanoparticles can lead to changes in their optical properties, hinder their dispersion in solvents or matrices, and negatively impact their stability and reactivity. The successful prevention of agglomeration indicates that the 22 nm stabilizing agent effectively maintained the dispersion and stability of the AuNPs, contributing to their uniformity and well-defined morphology.

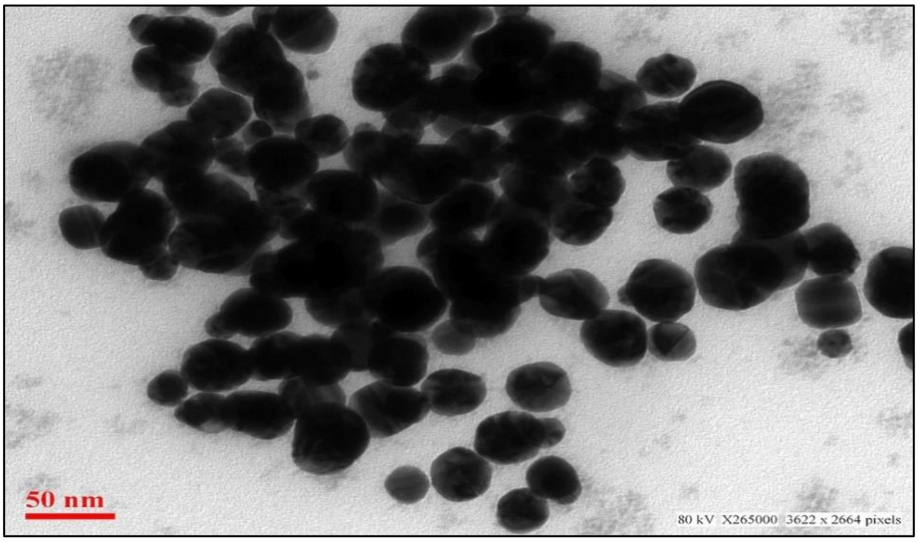

Fig.6 TEM micrograph of C-AuNPs.

4.1.3 Dynamic Light Scattering and Zeta-Potential Analysis

The DLS technique revealed an average hydrodynamic size of 65.12 nm for the C-AuNPs, considering the impact of the solvent layer (fig.7). It is noteworthy that the DLS measurement of C-AuNPs yielded a significantly higher size (65.12 nm) compared to the size measured by Transmission Electron Microscopy (TEM) (22 nm). This discrepancy arises due to the different nature of the two techniques in estimating particle size. TEM analysis is often conducted in dry conditions, which neglects the impact of the solvent layer surrounding the nanoparticles when they are dispersed. In this dry state, the TEM technique provides an estimate of the precise size of individual particles in their dehydrated form.

On the other hand, the DLS approach measures the hydrodynamic diameter of the nanoparticles, accounting for the effect of the surrounding solvent layer in their hydrated state. When nanoparticles are dispersed in a liquid medium, the solvent molecules form a layer around each nanoparticle, influencing its hydrodynamic behaviour. As a result of this solvent effect, the particles exhibit a larger hydrodynamic volume when analyzed using DLS. The difference between the measured sizes from TEM and DLS arises due to the influence of the solvent layer in the dispersed state. The DLS measurement includes the contribution of the solvent layer, leading to the observed larger hydrodynamic size of C-

AuNPs. This distinction is crucial to consider while interpreting the particle size data obtained from different techniques.

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	65.12	Peak 1:	78.03	100.0	29.78
PdI:	0.195	Peak 2:	0.000	0.0	0.000
Intercept:	0.895	Peak 3:	0.000	0.0	0.000
Result quality	Good				

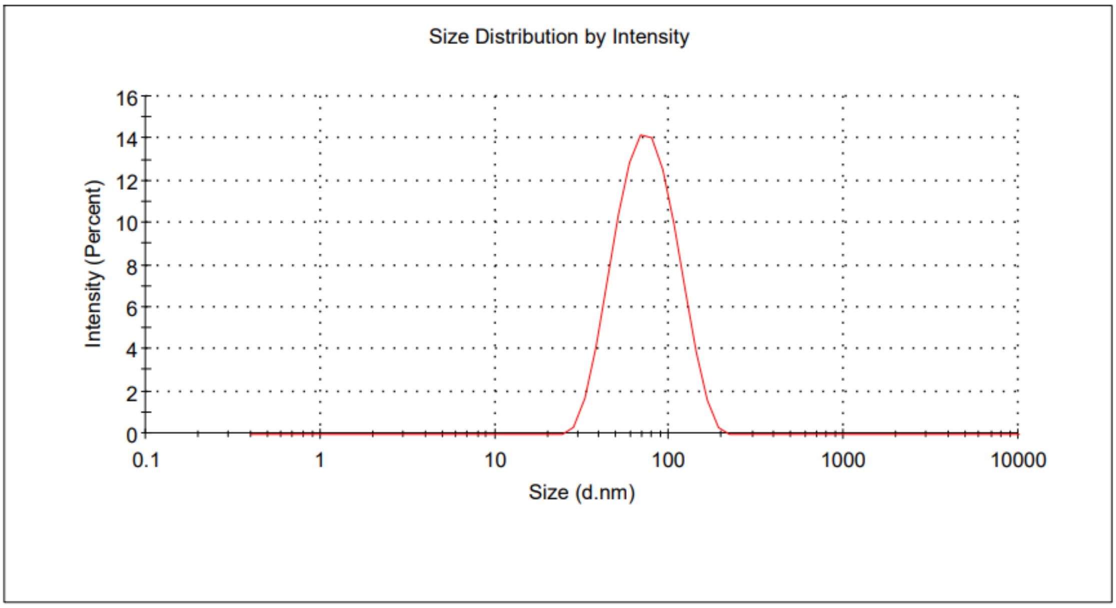

Fig.7 Dynamic Light Scattering Analysis Depicting the Average Particle Size and Particle Size Distribution Profile of C-AuNPs.

Zeta potential estimation is another crucial characterization method utilized in this study to determine the surface charges of nanoparticles. The zeta potential provides valuable insights into the electrostatic interactions between nearby particles in a dispersion that possess similar charges. This parameter serves as a key indicator of the stability and long-term viability of colloidal dispersions.

In the case of C-AuNPs, the zeta potential value was measured to be -14.6 mV, as demonstrated in fig.8. This negative zeta potential indicates a significant level of stability for the synthesized nanoparticles. When nanoparticles have a zeta potential value within a certain range, typically above ±30 mV, they exhibit strong electrostatic repulsion, preventing them from aggregating or coalescing in the dispersion. The presence of a relatively high negative zeta potential, such as -14.6 mV in this case, suggests that the C-AuNPs are well dispersed and repel each other electrostatically, leading to a stable colloidal suspension.

The stability of nanoparticles in colloidal dispersions is of paramount importance, as it directly influences their performance in various applications. Stable nanoparticles are less prone to aggregation and sedimentation, which can lead to diminished reactivity and hinder their suitability for practical use. The negative zeta potential value observed for the C-AuNPs demonstrates effective electrostatic repulsion between particles, preventing them from approaching closely and forming aggregates, thus ensuring the long-term stability of the dispersion.

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-14.6	Peak 1:	-14.6	100.0	11.5
Zeta Deviation (mV):	11.5	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	7.54	Peak 3:	0.00	0.0	0.00
Result quality	Good				

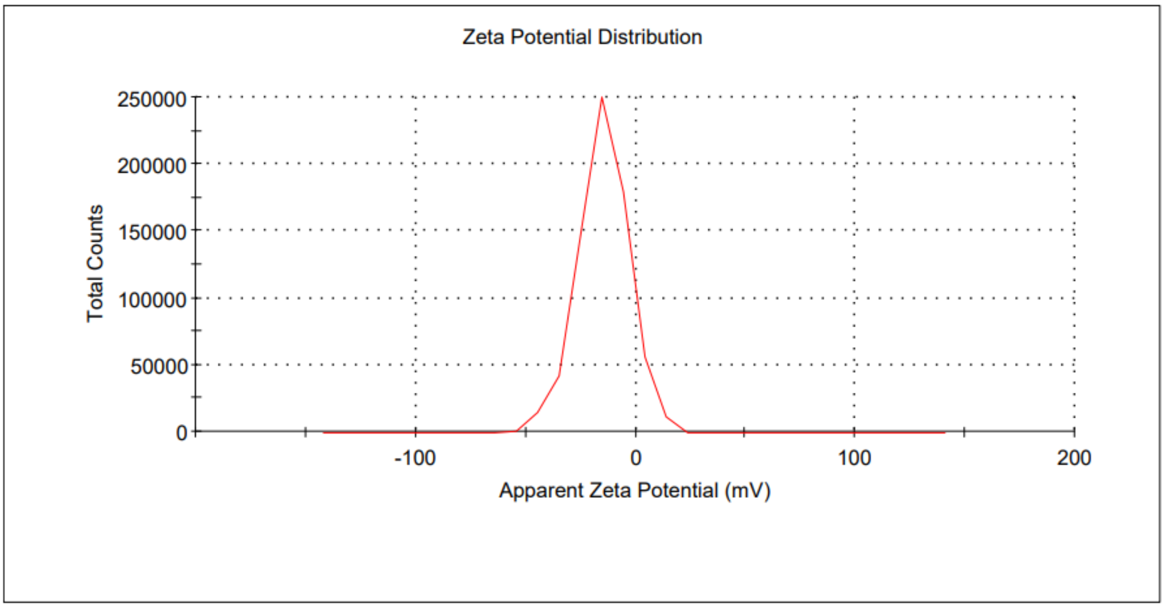

Fig.8 Zeta potential displays the colloidal stability of nanoparticles (C-AuNPs).

4.2 Antibacterial Activity Analysis of C-AuNps

Phosphate-buffered saline (PBS) was employed as a control for assessing the antibacterial effects of C-Aq, C-AuNPs, and pure levofloxacin in our experiments. The bacterial strains tested encompassed Gram-negative *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15692), and the Gram-positive *Bascillus subtilis* (MTCC 8114) strain. In the subsequent phase of our study, we introduced C-Aq, C-AuNPs, and pure levofloxacin into agar, leading to a marked suppression of bacterial growth, as demonstrated in Table 1. Our findings revealed the heightened efficacy of C-AuNPs in comparison to the pure levofloxacin antibiotic. This distinction was evident through the notably larger zones of inhibition observed in plates treated with C-AuNPs, surpassing the inhibitory zones noted in both pure levofloxacin and C-Aq treated samples. Notably, even at lower concentrations, C-AuNPs exhibited superior effectiveness against the targeted bacterial strains compared to pure levofloxacin alone. Across various tests, C-AuNPs consistently outperformed both C-Aqs and levofloxacin. In addition to their recognized antibacterial properties, C-AuNPs could potentially induce damage to bacterial DNA, likely through direct interactions that inhibit processes such as DNA unwinding during transcription. Detailed measurements of the zones of inhibition can be found in Table 1.

Table 1. Zone of Inhibition recorded for C-AuNPs, C-Aq and pure levo against gram-negative and gram-positive bacterial strains.

Zone of Inhibition (mm)			
Sample	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bascillus subtilis*
PBS (Control)	-	-	-
C-AuNPs	28 ± 0.12	23 ± 0.9	21 ± 1.6
C-Aq	13 ± 0.15	11 ± 0.5	20 ± 1.1
Levofloxacin	18 ± 0.16	19 ± 0.5	13 ± 1.3

4.3 Determination of Minimal Concentration of C-aq and C-AuNPs against gram negative and gram-positive bacterial strains

The findings from the agar well-diffusion assay provided insights into the Minimum Inhibitory Concentrations (MICs) for the bacterial strains under study, as depicted in Fig.9. Notably, C-AuNPs exhibited MICs of 94.97 µg/well, 26 µg/well, and 67.48 µg/well against *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis,* respectively. On the other hand, C-Aqs displayed MICs of 250 µg/well, 73 µg/well, and 85.53 µg/well against the same respective strains. Comparatively, levofloxacin's MICs were determined to be 162.96 µg/well, 40.56 µg/well, and 69.6 µg/well *for Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis.*

The outcomes of the antibacterial evaluations showcased an enhanced efficacy of C-AuNPs when associated with AuNPs, especially evident against Gram-negative microorganisms. This trend aligns with the observations by Shamaila et al., who reported increased effectiveness of chemically synthesized gold nanoparticles against Gram-negative enteric pathogens compared to Gram-positive counterparts. The divergence in structural attributes between Gram-negative and Gram-positive microorganisms likely contributes to this discrepancy. The ability of AuNPs to effectively bind to cell membranes and outer substrates of pathogens, driven by their diminutive size and exceptional penetration capabilities, is noteworthy. These nanoparticles could also induce membrane disruptions that remain irreparable. Another advantage of employing AuNPs as carriers lies in their expansive surface area, facilitating the transportation of a significant antibiotic load.

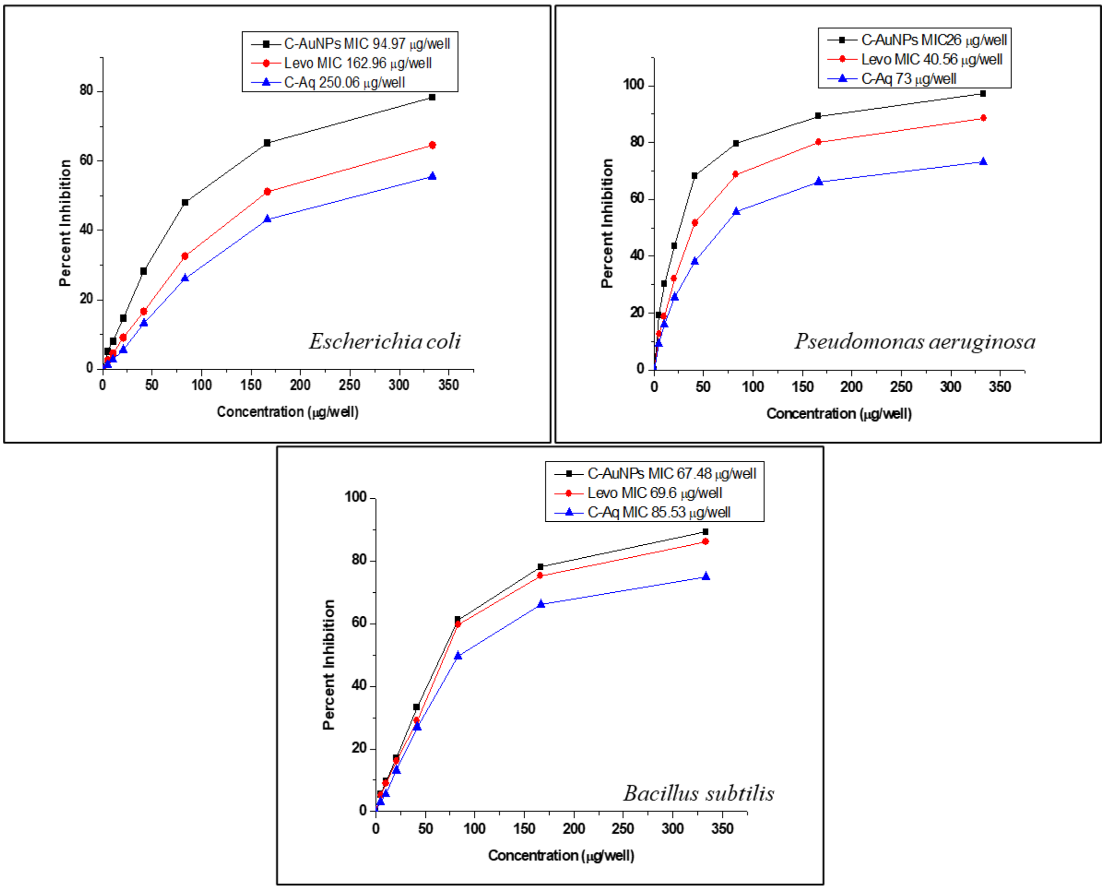

Fig.9 MIC of C-AuNps, C-Aq and antibiotic levofloxacin tested against gram negative (*Escherichia coli, Pseudomonas aeruginosa*) and gram positive (*Bacillus subtilis*) bacterial strains.

4.4 The anti-diabetic potential of C-AuNPs and C-Aq in contrast to standard inhibitor acarbose

The α-amylase inhibition assay was employed to evaluate the activity of the aqueous extract of C-Aq, aiming to deduce its potential anti-diabetic effects. Acarbose was utilized as a standard inhibitor, yielding an IC50 value of 45.7 µg/mL. In contrast, when the concentrations of 5, 10, 20, 40, 80, and 160 µg/mL were applied, the aqueous extract of C-Aq demonstrated α-amylase inhibition rates of 4.2%, 8.23%, 16.1%, 38.2%, 76.1%, and 92%, respectively. Similarly, C-AuNPs displayed inhibition rates of 8.3%, 12.2%, 28.1%, 56%, 90.4%, and 96% for the same respective concentrations. The percentage inhibition results indicated that C-AuNPs (with an IC50 of 35.6 µg/mL) exhibited robust inhibitory activity, surpassing both the aqueous extract of C-Aq (with an IC50 of 52.6 µg/mL) and the standard inhibitor acarbose (with an IC50 of 45.7 µg/mL), as depicted in Fig.10

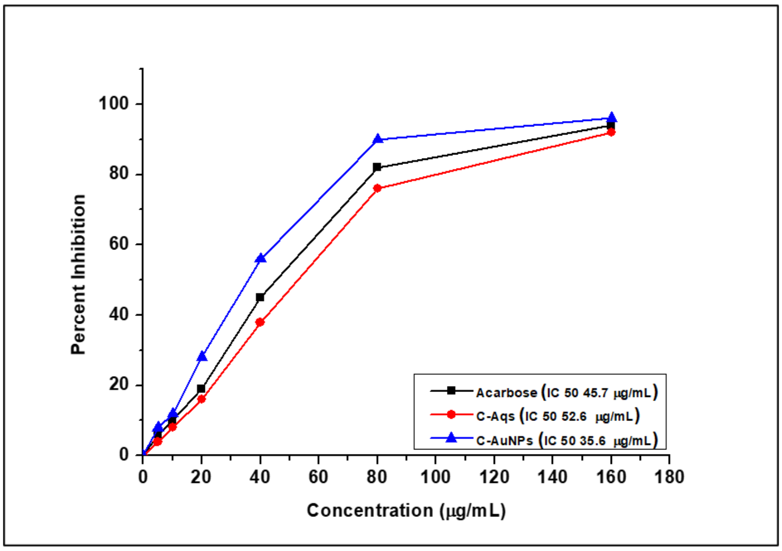

Fig.10 α-amylase inhibition potential of C-AuNPs and C-Aq

5. CONCLUSION

We successfully synthesized C-AuNPs (gold nanoparticles) using *Citrus sinesis* phytoconstituents through an eco-friendly approach. These nanoparticles exhibited characteristics of monodispersity, spherical morphology, and notable stability. Our investigation encompassed the assessment of the antibacterial potential of these synthesized C-AuNPs against a spectrum of Gram-negative and Gram-positive bacterial strains. Levofloxacin, a standard antibiotic, was used as a comparative benchmark. Interestingly, our findings revealed that while C-Aq exhibited antibacterial activity, the comparison among C-Aq, C-AuNPs, and levofloxacin highlighted the significantly enhanced antibacterial potency of C-AuNPs, even at lower concentrations.

Furthermore, our research delved into the evaluation of antidiabetic potential, specifically focusing on α-amylase inhibition. The outcomes illuminated the inherent α-amylase inhibitory activity of C-Aq. Moreover, the synergistic effect of C-AuNPs, synthesized via Citrus sinesis phytoconstituents, markedly amplified this inhibitory potential. This enhancement signifies the potential of C-AuNPs to augment the antidiabetic efficacy of C-Aq. The present study not only established a greener approach to fabricate C-AuNPs utilizing *Citrus sinesis* constituents but also revealed their multifaceted capabilities. These findings holds potential for the development of advanced antibacterial formulations.

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