A DISSERTATION ON

Green synthesis, characterization, and antimicrobial activity of gold nanoparticles synthesized by using *Citrus Limon* (Lemon) seed extract

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Khadijah Zakir**, a student of M.Sc. Microbiology (IV semester), Integral University has completed her four months dissertation work entitled ***"Green synthesis, characterization, and antimicrobial activity of gold nanoparticles synthesized by using Citrus Limon (lemon) seed 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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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Khadijah Zakir**, 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 ***"Green synthesis, characterization, and antimicrobial activity of gold nanoparticles synthesized by using Citrus Limon (lemon) seed extract"*** is, therefore, being forwarded for acceptance in partial fulfillment of the requirements for the degree award of the student of M.Sc. Microbiology (IV semester), Department of Biosciences, Integral University, Lucknow, (U.P).

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Khadijah Zakir **Date**

LIST OF ABBREVIATIONS

AuNPs	Gold nanoparticles
LVN	Levofloxacin
BRN	Bromelain
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	Nanometer

Introduction

Nanotechnology

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

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

Nanotechnology encompasses the fabrication and application of chemical, physical, andbiological systems at scales ranging from individual molecules or atoms to submicron dimensions, and also the integration of these resulting nanomaterials into larger systems. It has the potential to change our perspectives and expectations and provide us with the capability to resolve global issues. The discovery and use of carbon nanomaterials have allowed the introduction of many new areas of technology in nanomedicine, biosensors, and bioelectronics. In recent years, nanotechnology has emerged as a multidisciplinary field, in which gaining a fundamental understanding of the electrical, optical, magnetic, and mechanical properties of nanostructures promise to deliver the next generation of functional materials with wide-ranging applications **[3].**

Nanoparticles

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

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

Types of nanoparticles

1. Metallic nanoparticles -These nanoparticles have been used in drug delivery, especially in the treatment of cancer and in biosensors. Amongst various metals, silver, gold, and selenium nanoparticles are prime for medical use **[6].**

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

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

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

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

Platforms of nanoparticles

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

1) **Liposomes-** Liposomes are the first platform for NPs. In 1965, liposomes were described as a model of the cellular membrane **[20].** After that liposomes were used for genetic and drug delivery. Liposomes are vesicles in spherical shapes which contain lipids of single or multiple bilayer structures that can assemble themselves in aqueous systems **[21].** Liposomes can be used for targeting ligands to upsurge the buildup of diagnostic and therapeutic agents with anticipated cells. Now, 12 liposome-based therapeutic drugs are clinically approved.

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

3) **Polymeric-** These NPs are formed from biocompatible and biodegradable polymers whichare used as a therapeutic carriers **[24].** Polymeric NPs are verbalized through block-polymers of diverse hydrophobicity **[25].** This NPs design is useful because of the slow and control released of drugs at required sites.

4) **Quantum dots-** QDs are semiconductor particles, and their size is

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

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

Review of literature

Citrus limon: General introduction

Lemon (*Citrus limon* (L.) Burm.) a yellow or pale yellow prolate fruit with five to 10 seeds, botanically a berry, is known throughout the world and is used in numerous foodways and cuisines. The juice of the fruit is commonly used as a foodingredient in both commercial and home recipes and is valued for its tart, tangy, and fresh character. The exceptionally fragrant rind is used as a garnish andflavoring and is a major source of commercial essential oil and aroma compounds. The lemon flower is sweetly scented, with an aroma like other citrus flowers. The essential oils from the flowers, twigs, and fruit can be distilled or otherwise extracted to obtain materials suitable for use in flavors and fragrances.

The lemon tree is a small, thorny tree that grows well throughout the tropical and subtropical regions of the world. Lemons are cultivated in modest amounts for home use almost everywhere that citrus trees can survive, but large-scale commercial production is limited to the subtropical regions since more humid tropical environments enhance pest and disease problems for both fruit and tree. Lemon trees grow to about 3–6 m and are sparsely foliaged. Compared to orange and grapefruit, the trees are rather cold-sensitive. Because of the lemon's lower sugar content, the fruit can freeze at temperatures that would not affect another citrus.

The lemons have existed for so long that their true origin is not known, although experts believe it was probably a hybrid of the citron (*C. medica*). The first recorded habitat of the lemon was Southeast Asia (Myanmar, and southern China); from there, it was introduced to Persia and the Middle East, where it was well established by the twelfth century. Around 1150, Arabs took the lemon to Spain and North Africa. The lemon traveled from the Mediterranean to the New World with Columbus on his second voyage in 1493. Lemon culture spread from Haiti through the West Indies and then to South and North America. Plantings existed in Florida by the 1500s and were established in the mission gardens of California and Baja California by the mid-eighteenth century.

Figure 1. *Citrus limon*

Classification

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Rutales
- Family: Rutaceae
- Genus: Citrus
- Species: *Citrus limon*

Application of *Citrus Limon*

- High blood pressure. Middle-aged women who drink more lemon juice or eat more lemons seem to have lower systolic blood pressure (the top number). But they don't seem to have lower diastolic blood pressure (the bottom number).

- Heart attack. In people who are in the hospital after a heart attack, early research shows that inhaling the scent of lemon might reduce blood pressure and anxiety, and possibly help the heart recover.
- Morning sickness. A small study shows that placing a lemon essential oil on a cotton ball and inhaling the scent might reduce nausea in pregnant women.
- Problems making saliva due to radiation therapy.
- Decreasing swelling.
- Infection of theairways.
- Hay fever.
- Kidney stones.
- Increasing urine.
- An inner ear disorder is marked by dizziness, hearing loss, and ringing in theear (Meniere disease).
- Obesity.
- A disease caused by vitamin C deficiency (scurvy).
- The skin care, when applied to the skin

Figure 2. *Citrus limon* Seed

Nutritional Information about Lemon Seeds

It can be said that the nutrition contained in lemon seeds is similar to the one in the whole lemon fruit. it owns a good amount of antioxidants which mainly composedof vitamin *C. lemon* seeds is also surprisingly have the content of salicylic acid with the ability to relieve pain.

It also contains amino acids, just like what is owned by guava seeds with Health Benefits of Guava Seeds. Besides, lemon seeds also own a small amount of protein and fat which is beneficial for our health.

Health Benefits of Lemon Seeds

Here are some surprising health benefits of lemon seeds:

1. **Detoxifying property**

   Sometimes, we blend lemon along with the seeds so there's no way we can avoid consuming the seeds. But don't worry. It is okay to have lemon juice along with the seeds, as the seeds are a very good detoxifying property. Lemon seeds can clean the body from toxins, parasites, and other unwanted material. Well, it might be a little bit bitter, but it is okay as you'll get the health benefits of lemon seeds.

2. **Relieve pain**

   Who knows lemon seeds own some powerful health benefits of lemon seeds? Well, lemon seeds contain salicylic acid, one of the main components of aspirin. As we know, many people rely on aspirin to heal a headache and other kinds of pain. Therefore, consuming lemon seeds are known to be beneficial for relieving some ache and pain in an easy, safe, and natural way.

3. **Eliminate threadworm**

   It is common for children to have threadworm infections. However, we cannot let the common thing happen that way. Threadworm not only harms children's digestion but is also dangerous to children's growth.

   In this case, lemon seeds can offer a natural remedy to eliminate threadworm with detoxifying property owned by lemon seeds. We can simply crush some lemon seeds and boil them with water or milk. Give it to the children and we canmake sure the threadworm will stomp off the bowel quickly.

4. **Prevent candidiasis**

   One of the health benefits of lemon seeds is the ability to prevent candidiasis. Candidiasis is a fungal infection in the digestive tract because of Candida. In thiscase, the antifungal property in lemon seeds can fight the infection effectively. The other natural ingredient with a powerful ability in fighting candidais the Health Benefits of Water and Apple Cider Vinegar.

5. **Nourish skin**

   Not only lemon which owns Benefits of Lemon Water For Health, and Beauty. Just like the fruit which is praised as the powerhouse of vitamin C, lemon seeds also contain plenty of vitamin C as a beneficial antioxidant. It makes lemon seeds beneficial to maintaining healthy and youthful skin. This is also the reason why wecan find lemon seeds in some skin care products. As the seeds produce essential oil which nourishes and moisturize the skin very well.

6. **Treat acne**

   Lemon seeds produce essential oil with plenty of health benefits of lemon seeds, suchastheir antibacterial property. This way, the essential oil from lemon seeds is very effective to treat acne. As we know, the main culprit of acne is a bacterial infection.

7. **Give pleasant scent**

   Not only give the health benefits of lemon seeds in a form of beneficial nutrients, but lemon seeds also have a lemon-like scent that can be used for many things. It can be used to give natural fragrance to the cosmetic product, or it can also be used as aromatherapy to elevate our mood.

   In addition, it can also be used as a room freshener to get rid of mosquitos. Weknow, t h a t mosquito hates fresh lemon scents. The other natural ingredient with a lemon-like scent is verbena leaves, which own the Health Benefits of Verbena Leaves**.**

8. **Treat urinary tract infection**

   The consumption of lemon seeds is surprisingly baled to cure urinary tract infections as the seeds own the powerful antibacterial property. The lemon seeds extract can even fight antibiotic-resistant urinary tract infections. Not only the extract, but we can also take the whole seeds to treat urinary tract infections.

9. **Relieve nail fungus**

   Lemon seeds extract can be used to fight the fungus causing athlete’s foot and nail fungus, the fungal infection around toes and toenails. It can be used as the replacement of tea tree oil with its Health Benefits of Tea Tree Oil on Skin which effectively reduce the itching and burning of skin around the toes.

10. **Antibacterial spray**

    Lemon seeds extract can also be used as an antibacterial spray which can be used for your face, hands, and your eating utensils, and even you can add it in your final rinse for your laundry to make sure your laundry is free from any bacteria. It is simple, and safe, with many health benefits of lemon seeds.

    The other health benefits of lemon seeds are:

11. Treat eczema
12. Improve immune system
13. Prevent yeast infection
14. Kill pathogenic organisms in carpet
15. Disinfecting hot tubs
16. Reduce infectious diseases in animal feed

Nanotechnology

Nanotechnology is a field of research and innovation concerned with building 'things' - generally, materials and devices - on the scale of atoms and molecules. A nanometer is one-billionth of a meter: ten times the diameter of a hydrogen atom. The diameter of a human hair is, on average, 80,000 nanometers. At such scales, the ordinary rules of physics and chemistry no longer apply. For instance, materials' characteristics, such as their color, strength, conductivity, and reactivity, can differ substantially between the nanoscale and the macro. Carbon 'nanotubes' are 100 times stronger than steel but six times lighter.

Nanotechnology improves existing industrial processes, materials, and applicationsby scaling them down to the nanoscale to ultimately fully exploit the unique quantum and surface phenomena that matter exhibits at the nanoscale. Nanotechnology is hailed as having the potential to increase the efficiency of energy consumption, help clean the environment, and solve major health problems.It is said to be able to massively increase manufacturing production at significantly reduced costs. Products of nanotechnology will be smaller, cheaper, lighter yet more functional, and require less energy and fewer raw materials to manufacture, claim nanotech advocates.

Nanoscience breakthroughs in almost every field of science and nanotechnologies make life easier in this era. Nanoscience and nanotechnology represent an expanding research area, which involves structures, devices, and systems with novel properties and functions due to the arrangement of their atoms on the 1–100 nm scale. The field was subject to growing public awareness and controversy in the early 2000s, and in turn, the beginnings of commercial applications of nanotechnology. Nanotechnologies contribute to almost every field of science, including physics, materials science, chemistry, biology, computer science, and engineering. Notably, in recent years nanotechnologies have been applied to human health with promising results, especially in the field of cancer treatment. To understand the nature of nanotechnology, it is helpful to review the timeline of discoveries that brought us to the current understanding of this science. Thisreview illustrates the progress and main principles of nanoscience and nanotechnology and represents the pre-modern as well as modern timeline era of discoveries and milestones in these fields.

Application of Nanotechnology

Nanotechnology and nanomaterials can be applied in all kinds of industrial sectors.They are usually found in these areas.

Electronics

Carbon nanotubes are close to replacing silicon as a material for making smaller, faster, and more efficient microchips and devices, as well as lighter, more conductive, and stronger quantum nanowires. Graphene's properties make it an ideal candidate for the development of flexible touchscreens.

Energy

A new semiconductor developed by Kyoto University makes it possible to manufacture solar panels that double the amount of sunlight converted into electricity. Nanotechnology also lowers costs, produces stronger and lighter wind turbines, improves fuel efficiency and, thanks to the thermal insulation of some nano components, can save energy.

Biomedicine

The properties of some nanomaterials make them ideal for improving the early diagnosis and treatment of neurodegenerative diseases or cancer. They can attack cancer cells selectively without harming other healthy cells. Some nanoparticles have also been used to enhance pharmaceutical products such as sunscreen.

Environment

Air purification with ions, wastewater purification with nanobubbles, or nanofiltration systems for heavy metals are some of its environmentally friendly applications. Nanocatalysts are also available to make chemical reactions more efficient and less polluting.

Food

In this field, nano-bio sensors could be used to detect the presence of pathogens in food or nanocomposites to improve food production by increasing mechanical and thermal resistance and decreasing oxygen transfer in packaged products.

Textile

Nanotechnology makes it possible to develop smart fabrics that don't stain nor wrinkle, as well as stronger, lighter, and more durable materials to make motorcycle helmets or sports equipment.

Advantages of Nanotechnology

Many potential applications and advantages include:

- Advances in disease treatments, such as cancer
- Better imaging and diagnostic equipment
- Energy-efficient products such as fuel and solar cells
- Improvements in manufacturing that allow for durable, light-weight, efficient production tools
- Improved electronic devices, including transistors, LED and plasma displays,and quantum computers
- Nanorobots can be used to rebuild the ozone layer, clean polluted areas, and lessen dependence on non-renewable energy sources.

Understanding, shaping, and combining matter at the atomic and molecular scale is called nanotechnology. Nanotechnology encompasses science, medicine, engineering, computing, and robotics at this scale, called the nanoscale. Nanotechnology offers the potential for new and faster kinds of computers, more efficient power sources, and life-saving medical treatments. Potential disadvantages include economic disruption and possible threats to security, privacy, health, and the environment.

Manufacturing Advantages of Nanomaterials

Nanotechnology is already making new materials available that could revolutionize many areas of manufacturing. For example, nanotubes and nanoparticles, which are tubes and particles only a few atoms across, and aerogels, materials composed of very light and strong materials with remarkable insulating properties, could pave the way for new techniques and superior products. In addition, robots that are only a few nanometers in length called nanobots, and nano factories could help construct novel materials and objects.

Energy and Electronic Advantages

Nanotechnology may transform how we obtain and use energy. Nanotechnology will likely make solar power more economical by reducing the cost of constructing solar panels and related equipment. Energy storage devices will become more efficient as a result. Nanotechnology will also open up new methods of generating and storing energy.

The field of electronics is set to be revolutionized by nanotechnology. Quantum dots, for example, are tiny light-producing cells that could be used for illumination or purposes such as display screens. Silicon chips can already contain millions of components, but the technology is reaching its limit; at a certain point, circuits become so small that if a molecule is out of place the circuit won't work properly. Nanotechnology will allow circuits to be constructed very accurately on an atomic level.

Medical Benefits of Nanotechnology

Nanotechnology has the potential to bring major advances in medicine. Nanobots could be sent into a patient's arteries to clear away blockages. Surgeries could become much faster and more accurate. Injuries could be repaired cell by cell. It may even become possible to heal genetic conditions by fixing the damagedgenes. Nanotechnology could also be used to refine drug production, tailoring drugs at a molecular level to make them more effective and reduce side effects.

In 2021, Nonwoven News reported that researchers at the University of Rhode Island have developed a smart bandage that can detect and monitor infection in wounds using single-walled carbon nanotubes. The nanotubes can identify infections by detecting concentrations of hydrogen peroxide. The bandage is monitored wirelessly with a miniature wearable device and transmitted to a smartphone to alert the patient or a health care provider when an infection is detected.

Environmental Effects and Economic Issues

Some of the more extravagant negative future scenarios have been debunked by experts in nanotechnology. For example, the so-called "gray goo" scenario, where self-replicating nanobots consume everything around them to make copies of themselves, was once widely discussed but is no longer considered to be a credible threat. It is possible, however, that there will be some negative effects on the environment as potential new toxins and pollutants may be created by nanotechnology.

It is likely that nanotechnology, like other technologies before it, will cause majorchanges in many economic areas. Although products made possible by nanotechnology will initially be expensive luxury or specialist items, once availability increases, more and more markets will feel the impact. Some technologies and materials may become obsolete, leading to companies specializing in those areas going out of business. Changes in manufacturing processes brought about by nanotechnology may result in job losses.

Nanotechnology raises the possibility of microscopic recording devices, which would be virtually undetectable. More seriously, it is possible that nanotechnology could be weaponized. Atomic weapons would be easier to create, and novel weapons might also be developed. One possibility is the so-called "smart bullet," a computerized bullet that could be controlled and aimed very accurately. These developments may prove a boon for the military; but if they fell into the wrong hands, the consequences would be dire.

Advantages of Green synthesis over Chemical synthesis

I. Green synthesis is simple and usually involves a one-pot reaction.

II. it is amenable to scale up.

III. the toxicity associated with hazardous chemicals are eliminated,

IV. green biological entities can be used as reducing and capping agents, and

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

Gold Nanoparticles

Properties of gold nanoparticles are different from their bulk form because bulk gold is yellowsolid, and is inert while gold nanoparticles are wine red solution and are reported to beanti-oxidant. Interparticle interactions and assembly of gold nanoparticle networks play a keyrole in the determination of the properties of these nanoparticles **[50]**. Gold nanoparticles exhibit various sizes ranging from 1 nm to 8 µm and they also exhibit different shapes such as spherical, sub-octahedral, octahedral, decahedral, icosahedral multiple twined, multiple twined, irregular shape, tetrahedral, nano triangles, nano prisms, hexagonal platelets, and nanorods.

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

Figure 3. Gold colloids of different sizes.

Gold Nanoparticles

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

Applications of Gold Nanoparticles

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

1. **Electronics** - Gold nanoparticles are designed for use as conductors from printable inks to electronic chips. As the world of electronics becomes smaller, nanoparticles are important components in chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip.

2. **Photodynamic Therapy** - Near-IR absorbing gold nanoparticles (including gold nanoshells and nanorods) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these

nanoparticles to eradicate targeted tumors. When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.

3. **Therapeutic Agent Delivery** - Therapeutic agents can also be coated onto the surface of gold nanoparticles. The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers).

   Sensors - Gold nanoparticles are used in a variety of sensors. For example, a colorimetricsensor based on gold nanoparticles can identify if foods are suitable for consumption. Other methods, such as surface-enhanced Raman spectroscopy, exploitgold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds. This strategy could also be used for the detection of proteins, pollutants, and other molecules label-free.

4. **Probes** - Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy. The scattered colors of gold nanoparticles are currently used for biological imaging applications. Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.

5. **Diagnostics** - Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also common in lateral flow immunoassays, a common household example being the home pregnancy test.

6. **Catalysis** - Gold nanoparticles are used as catalysts in several chemical reactions. The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a reaction (nitrogen oxides). Gold nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry **[52].**

Other applications of gold nanoparticles are listed below:

- As an antibiotic, anti-fungal, and anti-microbial agent when added to plastics, coatings, nanofibers, and textiles
- In nanowires and catalyst applications
- In therapeutic agent delivery
- To connect resistors, conductors, and other elements of an electronic chip
- In photodynamic therapy - When light is applied to a tumor containing goldnanoparticles, the particles rapidly heat up, killing tumor cells
- In various sensors, e.g. colorimetric sensor with gold nanoparticles can identify if foods are suitable for consumption
- As substrates to enable the measurement of vibrational energies of chemicalbonds in surface-enhanced Raman spectroscopy
- The scattered colors of gold nanoparticles are currently used for biologicalimaging applications
- Gold nanoparticles are quite dense, thus allowing them to be used as probesfor transmission electron microscopy
- To detect biomarkers in the diagnosis of cancers, heart diseases, and infectious agents
- As catalysts in several chemical reactions
- For fuel cell applications.

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 ofmaterial 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 ofthe "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 containingthe 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.

Objectives

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Objectives

- Synthesis of AuNPs by using *Citrus limon* Seed extract.
- Characterization of synthesized Au-NPS by UV- vis spectroscopy, FTIR, TEM, DLS, and zeta potential.
- Antibacterial activity of synthesized AuNPs.

Materials and method

Materials

Tetra chloroauric acid ($HAuCl_4$) was purchased from Sigma Aldrich. Phosphate buffer salts (Na_2HPO_4) and (NaH_2PO_4) were purchased from HIMEDIA. Double distilled waterhas been used as an aqueous medium for all experiments. All buffers were filtered with 0.2µm filter paper immediately after they were prepared.

Method

1- Preparation of *Citrus limon seed* extract

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

2- In vitro synthesis of AuNPs

In vitro synthesis of AuNPs 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 0.48ml of freshly prepared lemon seed aqueous extract. This extract was used as a source for the synthesis of AuNPs and served as a reducing agent and provided stability to particles. The extract reduces Au (III) to Au (II) anions which were further reduced to form monodispersed, spherical Gold Nanoparticles of different sizes. On completion of the reaction, the synthesized Gold Nanoparticles were centrifuged for 5 minutes at 5000 rpm. The supernatant and the pellet were separated with the help ofa0.2µm filter. This was followed by the characterization of AuNPs using the technique UV-vis Spectroscopy.

Results

Results-

Figure 4. Synthesized Gold Nanoparticles

Characterization of Gold Nanoparticles

UV- vis Spectroscopy

The absorption spectra of AuNPs 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.

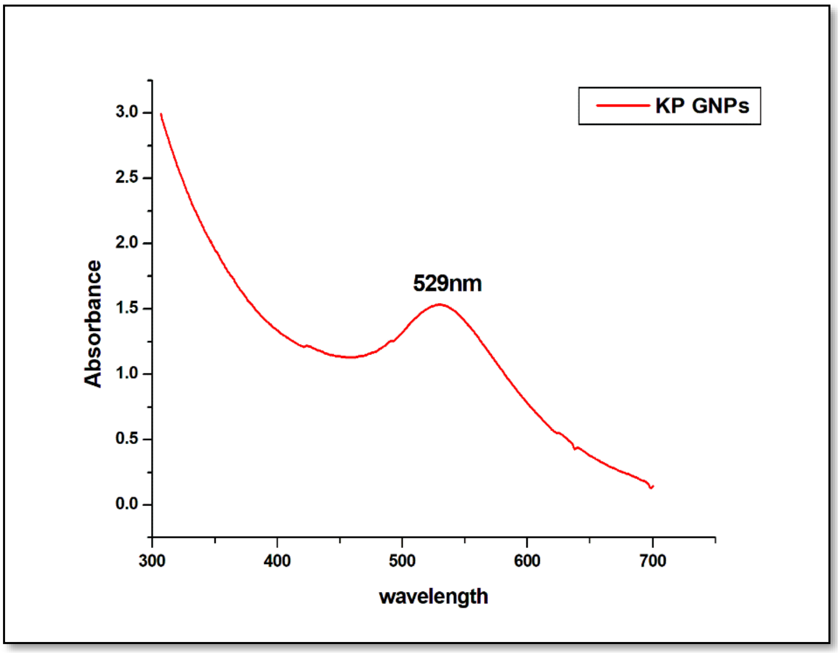

Figure 5. UV-Vis Spectra of synthesized Gold nanoparticles showing a peak at 529 nm.

UV-visible absorption spectra of gold nanoparticles exhibit a surface Plasmon resonance peak at 529nm the broadness and intensity show that the particle is small in size. If gold nanoparticles are below 2 nm in diameter, this Plasmon peak disappears due to the change in electronic structure that occurs with quantum confinement.

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DLS (Dynamic Light Scattering)

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

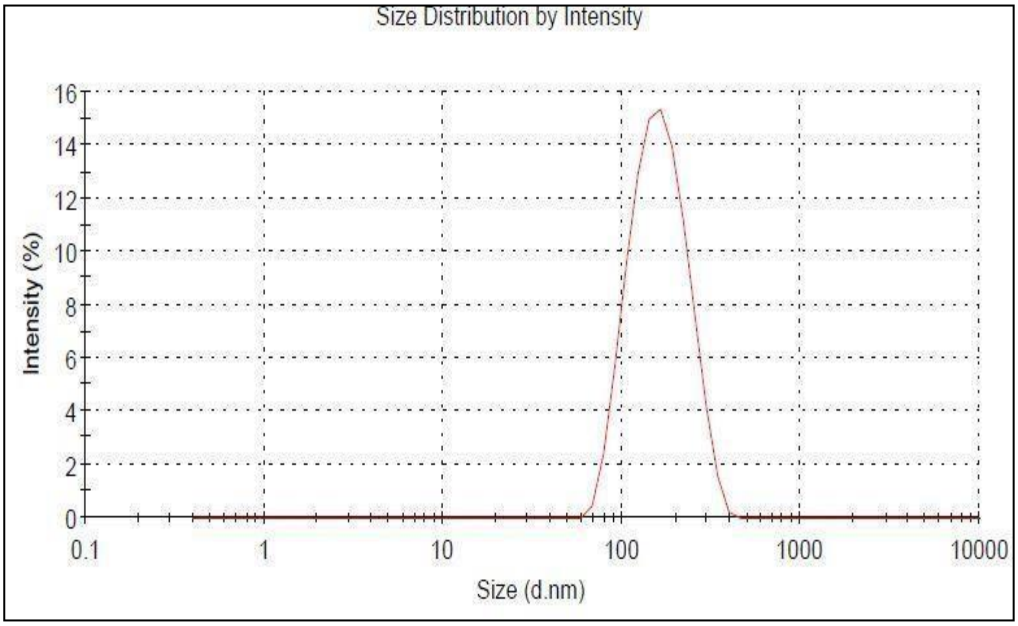

Figure 6. Size distribution of colloidal AuNPs (size distribution by intensity 139 d. nm).

Zeta Potential

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

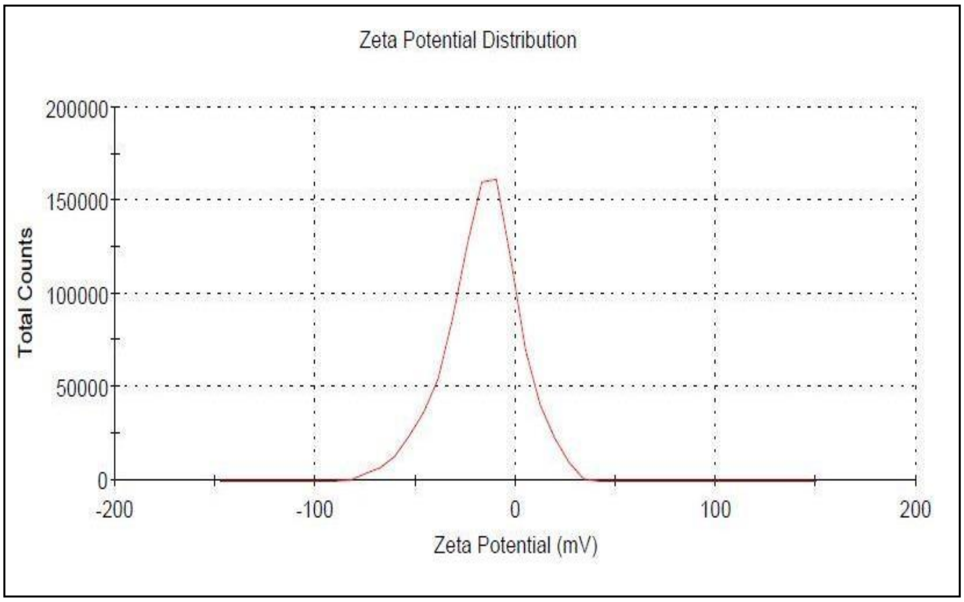

Figure 7. Zeta potential graph for AuNPs showing at -19 mV**.**

FTIR

FTIR spectroscopy was performed to determine the potential functional groups in the synthesized GNPs (Figure 8). The spectra of GNPs showed characteristic absorption peaks at 1639 cm−1 corresponding to C=O groups. A medium-wide shoulder, corresponding to amide I linkage and amide II band, was observed at 1455 cm−1 due to carboxyl stretch and N-H twist in the amide bond of the proteins, which were capped, or surface modified on the GNPs. The peak at 3285 cm−1 was due to the N–H stretch vibration, which depends on the strength of hydrogen bonding rather than backbone confirmation. In addition, ether and alcohol groups (C-O-C/C-OH) C-O stretching, and C-N (aliphatic amine) stretching vibration were observed at 1083 cm−1.

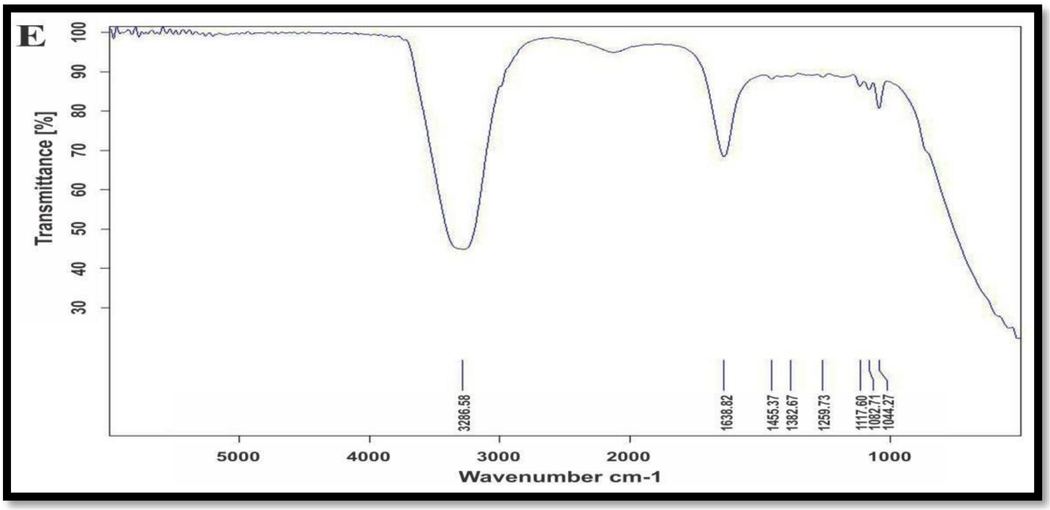

Figure 8. FTIR spectroscopy**.**

Transmission electron microscopy

The high-resolution image was acquired using the transmission electron microscope (TEM), which confirmed the average size of AuNPs as 26 ± 2 nm using a Gatan digital micrograph and showed by the spherical form of AuNPs. The TEM micrographs did not expose the agglomeration of the as-synthesized

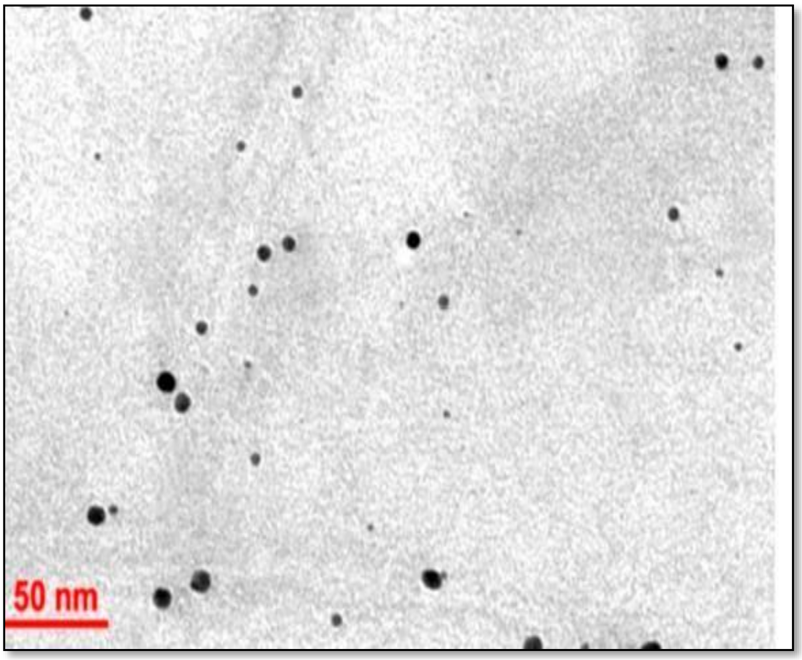

Figure 9. TEM analysis of AuNPs.

Antibacterial screening

The antibacterial action of synthesized AuNPs 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 AuNPs was evaluated against normal strains of *Escherichia coli, M.luteus, Staphylococcus aureus, and K. pneumonia*. The antibacterial potential was confirmed by a clear zone of inhibition surrounding the inoculated region. The maximum zone of inhibition was found against Escherichia coli.

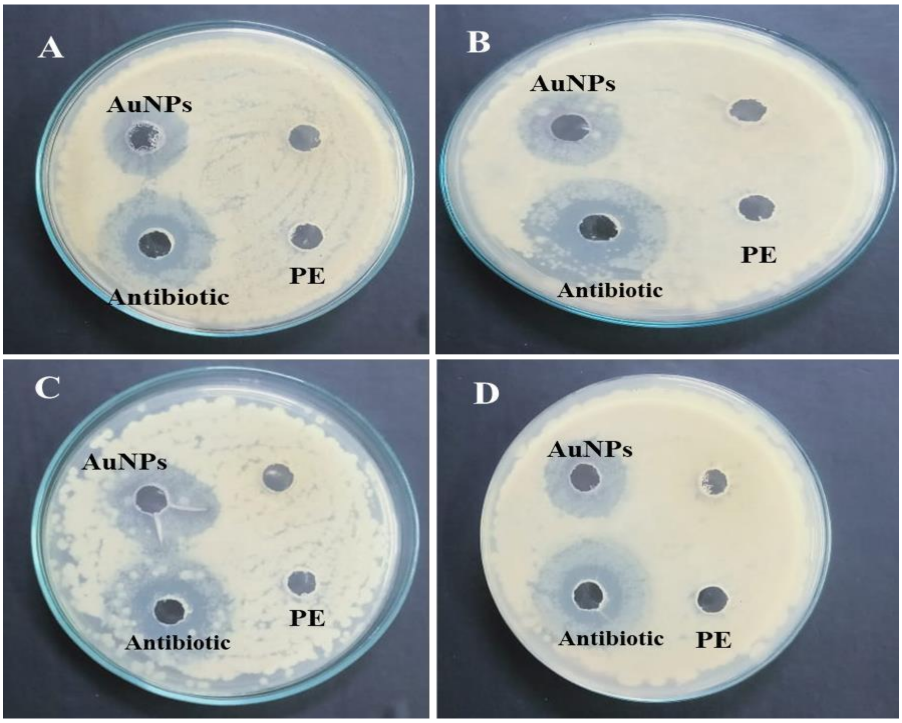

Figure 10. The synthesized AuNPs showed Activity against *S. aureus, M. luteus, E. coli, and K. pneumoniae* by causing a zone of inhibition.

Zone of inhibition

S. No.	Species	Gold Nanoparticle (Zone of Inhibition in mm)		
		AuNPs (50µg/well)	Antibiotic	Plant extract
1.	*Escherichia coli*	**16**	**24**	**7**
2.	*M. luteus*	**15**	**26**	**8**
3.	*Staphylococcus aureus*	**11**	**20**	**6**
4.	*K. pneumoniae*	**10**	**18**	**5**

The antibacterial studies with LG-AuNPs showed a profound antibacterial effect against both gram-positive and gram-negative strains. The results of the present study suggest that plants and silver in their nano form possess certain constituents with antibacterial properties that may be used as antibacterial agents in new drugs against common bacterial pathogens. The synthesized nanoparticle was active on all the organisms tested. The highest activity against the tested bacteria was obtained in *S. aureus*, *M. luteus, E. coli, and K. pneumoniae* gram-positive and negative bacteria.

MIC (Minimum Inhibitory Concentration)

We found that biogenic AuNPs synthesized by *citrus limon* aqueous seed extract show strong antibacterial activity against both Gram-positive and Gram-negative pathogenic bacterial strains. The MIC_{50} of AuNPs was evaluated against different pathogenic bacterial strains that included 14.5 µg/mL against *S. aureus*, 8.6 µg/mL against *M. luteus*, 6.063 µg/mL against *E. coli*, and 13.4 µg/mL against *K. pneumoniae*, indicating its broad-spectrum feature. However, we found that AuNPs were more effective against *E. coli* (Gram-negative) and *M. luteus* (Gram-positive) than other pathogenic strains. A thick peptidoglycan layer in Gram-positive bacteria prohibited the entry of AuNPs into the cytoplasm, and a higher AuNPs concentration is required to inhibit the growth of Gram-positive than Gram-negative bacteria.

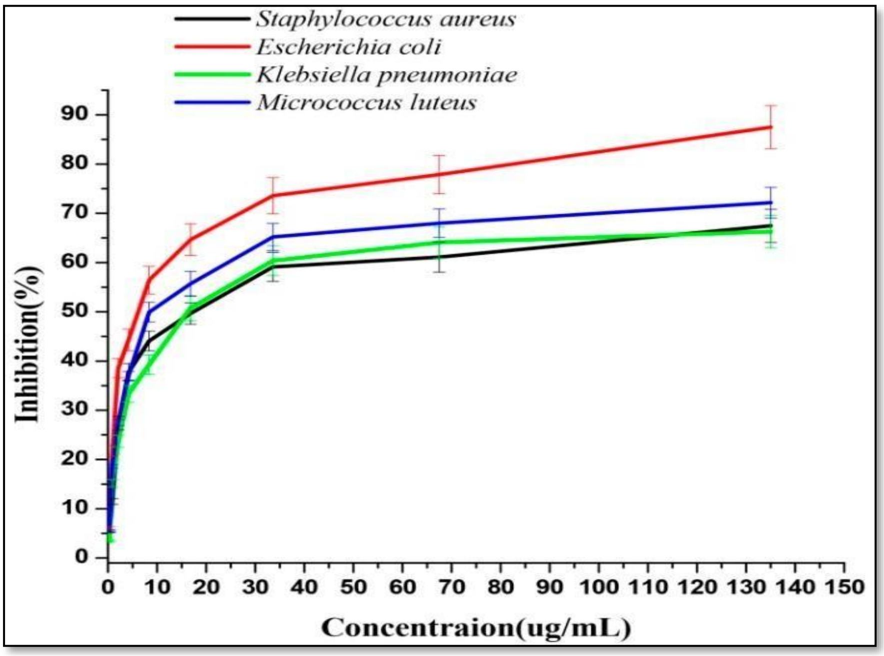

Figure 11. The antibacterial potential of AuNPs against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, and M. luteus.*

Discussion and conclusion

Discussion

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

In this study, *Citrus limon* seed aqueous extract was used for the synthesis of AuNPs. This extract was used as a source for the synthesis of AuNPs and served as a reducing agent and also provide stability to the particles. This plant extract reduced Au (III) to Au (II) anions which were further reduced to form monodispersed, spherical Gold NPs of different sizes.

The characterization of synthesized AuNPs was done by UV- visible spectroscopy and peak were found at 529nm, due to the surface plasmon resonance property of Gold Nanoparticles.

Therapeutic Analysis was performed for the AuNPs for antibacterial assay. A synthesized gold nanoparticle from *Citrus limon* seed aqueous extract shows broad inhibition against specific bacteria thereby making the synthesized gold nanoparticles a good antimicrobial agent.

Conclusion

Nanoparticle-based technologies cover different fields, ranging from environmental remediation, energy generation, development of potential drug molecules, etc. Nanoparticle characterization is necessary to establish an understanding and control of nanoparticle synthesis and applications. In this study, Gold Nanoparticles have been synthesized using *Citrus limon* seed extract. As a previous study, the plant has anticancer, antioxidant, antidiabetic, and antibacterial, activity.

These biogenic AuNPs exhibited significant dose-dependent antibacterial potentials. However, further investigations are warranted to assess the toxicity details and the mechanism associated with the antibacterial and anticancer action of the biosynthesized AuNPs. Nevertheless, the outcomes of the present study provide a broad AuNPs-based platform for various therapeutic applications soon.

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