A DISSERTATION ON

ONE POT SYNTHESIS OF GOLD NANOPARTICLES VIA *KINNOW MANDARIN* AQUEOUS EXTRACT AND ITS ANTI-BACTERIAL POTENTIAL ANALYSIS

SUBMITTED TO THE
DEPARTMENT OF BIOSCIENCES,
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT FOR THE DEGREE OF

MASTER OF SCIENCE

IN MICROBIOLOGY

BY

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M.SC MICROBIOLOGY

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LUCKNOW

UNDER THE SUPERVISION OF

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LUCKNOW

Declaration

I hereby declare that the present work on "**One Pot Synthesis Of Gold Nanoparticles Via Kinnow Mandarin Aqueous Extract And Its Anti-Bacterial Potential Analysis**" is a record of original work done by me under the guidance of **Dr. Zeeshan Rafi**, Lecturer, **Integral University**, during 2nd feb 2022 to 2nd june 2022,at Integral University, Lucknow. All the data which were provided in this were through our own original work.

I also declare that not any part of thesis has previsously been submitted to my University or any examining body for acquiring any diploma or degree.

Place: Integral University,Lucknow

Date:

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Alhusna Mujeeb**, student of M.Sc. Microbiology (IV semester), Integral University has completed her four months dissertation work entitled "**One pot synthesis of Gold Nanoparticles via *Kinnow mandarin* aqueous extract and its anti-bacterial potential analysis"** successfully. She has completed this work from 2nd feb to 2nd june 2022 under the guidance of **Dr. Zeeshan Rafi.** The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and bright future.

Dr. Snober. S.Mir

Head of department of bioscience

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the dissertation entitled **"One pot synthesis of Gold Nanoparticles via *Kinnow mandarin* aqueous extract and its anti-bacterial potential analysis."** has been submitted by **Ms. Alhusna Mujeeb**, in partial fulfillment of the requirements for the degree of **M.Sc. Microbiology** of the Integral University is an authentic record of the research work carried out by her under my supervision and guidance and that no part, thereof, has been presented before for any other degree.

She has successfully completed her project from Feb-02 to 02-June-2022. The dissertation was compulsory part of her M.Sc. Microbiology degree.

We wish her good luck and bright future.

Dr. Zeeshan Rafi

Lecturer,
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Before I present my work, I would like to gratefully acknowledge the contribution of all those people who have helped in the work described in this dissertation. I am going to try anyway, and if your name is not listed, it is rest assured that my gratitude is not less than for those listed below. Firstly, I would like to express my gratitude to my advisor ***Dr. Zeeshan Rafi*** *(Lecturer, Department of Biosciences) for the continuous support of my dissertation. His guidance helps me in all the time of dissertation work and writing of this report. I could not have imagined having a better advisor and mentor for my work.*

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Alhusna Mujeeb

ABBREVIATIONS

DLS	Dynamic Light Scattering
GNPs	Gold nanoparticle
HEPES	buffer
OD	Optical density
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
UV-Vis	UV Visible Spectroscopy
XRD	X-ray diffraction
KMAPE	*Kinnow mandarin* aqueous peel extract
K-AuNPs	Kinnow- gold nanoparticles
Nm	nanometer
PBS	phosphate buffer saline
ZP	zeta potential
MIC	Minimal Inhibitory Concentration

TABLE OF CONTENTS

INTRODUCTION

INTRODUCTION

The concern of nanotechnology and Nano science studies has overgrown in recent years, incomes the nickname ‘‘tiny science. Nanotechnology has been in the middle of the stage while technological advancements are taken into consideration and has elevated massive interest over time. Nanoparticles are the critical constructing additives of nanotechnology. NPs are debris among 1 and a hundred nm in length product of carbon, steel, steel oxides, or organic matter. Compared to their bulk counterparts, NPs have better floor charge, absorption, reactivity, floor area, sensitivity, stability, and strength.(Nanosci. 11 (9) (2016) 714–721) *Kinnow mandarin* (Citrus reticulata) which is a hybrid of two cultivars- ‘King’ and ‘Willow leaf’ mandarin is an important fruit among citrus fruits in terms of nutritive value as well as economic value. The fruit is mostly consumed as fresh fruit and juice extraction is equally done. The by - product after the juice extraction or fresh consumption is the peel and pomace. The kinnow peel is a rich source of various bioactive compounds and essential oils among which are polyphenols, carotenoids (lutein), pectin, naringin, antioxidants which have industrial importance. Polyphenols present in the peel such as naringin, limonene and flavonoids are said to have anti-inflammatory and anticancer properties (Tripoli et al., 2007; Xu et al., 2019).It is been suggested that the kinnow peel which constitutes 30-40% proportion of fruit is rich in bioactive compounds and has more polyphenol content as compared to other portions of the fruit (Lim et al., 2007).Thus, the important properties of citrus peel have prompted the researchers to explore the scope of this waste product as a valuable component for the processing industries (Babbar et al., 2011; Rafiq et al., 2018).The kinnow peel consists of two different tissues are found namely albedo (inner layer) and flavedo (outer layer). Albedo mainly constitutes various nutrients viz. minerals, sugars, proteins and fiber (Marin et al.,2007).On the other hand, flavedo constitutes essential oils which are useful for flavor and fragrance industry (Bejar et al., 2012).The essential oils present in the flavedo layer are rich in carotenoids, terpenes and linalool (Mondello et al., 2005) and it is suggested by various researchers that these bioactive compounds have antimicrobial properties and act as antioxidants (Tepe et al., 2006; Jayprakasha et al., 2008; Viuda-Martos et al., 2008).Thus, the fact that that the peel portion of the fruit has comparatively more phenolics and has higher potential for its useful ingredients than the fruit pulp (Deng et al., 2012; Goulas et al., 2012; Jabbar et al., 2015) has made the scope for the study of the constituents, their extraction and use for the industry.Kinnow peel extract against pathogenic strains of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and klebsiella pneumonia. Citrus (Citrus L.from Rutaceae) is one of the most popular world fruit crops,contains active that can

that can protect health. In addition to this, it provides an ample supply of vitamin C, folic acid, potassium and pectin. The contribution of citrus species in deterrence of life threatening diseases have been assessed (Proteggente et al., 2003; Gorinstein et al., 2004; Anagnostopoulou et al., 2006; Guimara˜es et al., 2009) and it has been reported that citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of promising biological properties due to their phenolic profile and antioxidant properties (Middleton and Kandaswami, 1994; Montanari et al., 1998; Samman et al., 1996). Citrus fruits are highly consumed worldwide as fresh produce, juice and most often the peel is discarded as waste which contains a wide variety of secondary components with substantial antioxidant activity in comparison with other parts of the fruit (Manthey and Grohmann, 2001). Global production of citrus fruit has significantly increased during the past few years and has reached 82 million tons in the years 2009–2010, of which oranges – commercially the most important citrus fruit accounts for about 50 million tons (USDA, 2010) and 34% of which was used for juice production, yielding about 44% peel as by- product (Li et al., 2006). Therefore, a large amount of peel is produced every year. Citrus peel, the primary waste, is a good source of molasses, pectin and limonene and is usually dried, mixed with dried pulps and sold as cattle feed (Bocco et al., 1998).In the field of nano medicine and nanotechnology, gold nanoparticles play essential role in enhancing pharmacological properties of drugs or extracts. It has also been stated that the plant extracts can reduce the inorganic salts into nanoparticles by capping and stabilize them. They are highly effective as antibacterial and anti-inflammatory agents. They enhance the efficacy and affinity of the drug and thus can manage various ailments.

Some of the applications of nanomaterials to biology or medicine are:

- Fluorescent biological labels
- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Probing of DNA structure
- Tissue engineering
- Tumour destruction via heating (hyperthermia)
- Separation and purification of biological molecules and cells
- MRI contrast enhancement

- Phagokinetic studies

So, the majority of nanoparticles applications in medicine is towards drug delivery. In biosciences, nanoparticles are replacing organic dyes that require high photo-Stability and high multiplexing capabilities.

REVIEW OF LITERATURE

Nanotechnology is interdisciplinary field of science which plays a dominant role in day to day life. Nanotechnology deals with the production, manipulation and use of materials at Nano scale. The term nano is derived from the Greek word “nanos” which means small and it is used as the prefix for one billionth part (10^{-9})According to American Society for Testing and Materials (ASTM international 2006), nanoparticles are those particles which have two or more than two dimensions and are in the size ranging from 1-100nm.(Alanazi et al., 2010). Nanoparticles are the essential building components of nanotechnology and are made up of carbon, metal, metal oxides, or organic matter. Compared to their bulk counterparts, Nanoparticles have higher surface charge, absorption, reactivity, surface area, sensitivity, stability and strength (K. Gudikandula, S. Charya Maringanti., 2016). The field of nanotechnology helps in the production of materials, mainly those for medical uses, where conventional approaches may reach their limits (S. Griffin, M.I. Masood, M.J. Nasim., 2018 E3).The history of nanomaterial’s usage in construction dates back to 4500 years ago. One of the oldest, richest and progressive cultures globally Egyptians realized the capabilities of nanomaterials 4000 years ago(Y.-M. Li, Y.-Y. Wang, B.-N. Cheng, 2017). Gold nanoparticles are the most compatible nanomaterial for preparation of engineered smart sensing devices. Nano is a prefix. Nano like micro, Pico etc., is used in front of a macroscopic unit to change its value by order of magnitude. When placed in front of words like science and technology, the meaning of nano is changed. Since experimental science and technology deals with material objects, it seems fair to say that Nano science and nanotechnology are science and technology concerning objects of nanometer dimension, which are atoms and molecules. Another definition of Nano science and nanotechnology can be achieved by focusing on the intrinsic properties of Nano scale objects as such, and on the possibility of using, manipulating, organizing them into assemblies in order to perform specific functions. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. There have been vast development in the field of nanotechnology in recent years, with numerous methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements. New applications of nanoparticles and nanomaterials are increasing rapidly. It seems that nanoscience may be the centre stage in many key future technologies because of recent efforts in fabricating these Nano sized structures into predefined superstructures.(Ankamwar et al., 2005).Recently, the British Standards Institution(Jeevanandam et al., 2018) proposed the following definitions for the scientific terms that have been used:

- Nanoscale: approx 1 to 1000 nm size range.
- Nanoscience: the science that deals with understanding theirsize and structure dependent properties and compare the emergence of individual atoms or molecules or bulk material related differences.

- Nanotechnology: deals with the manipulation and control of matter on the nanoscale dimension by using scientific knowledge of various industrial and biomedical applications.
- Nanomaterial: material with any internal or external structures on the nanoscale dimensions.
- Nanoparticle: nano-object with three external nanoscale dimensions.

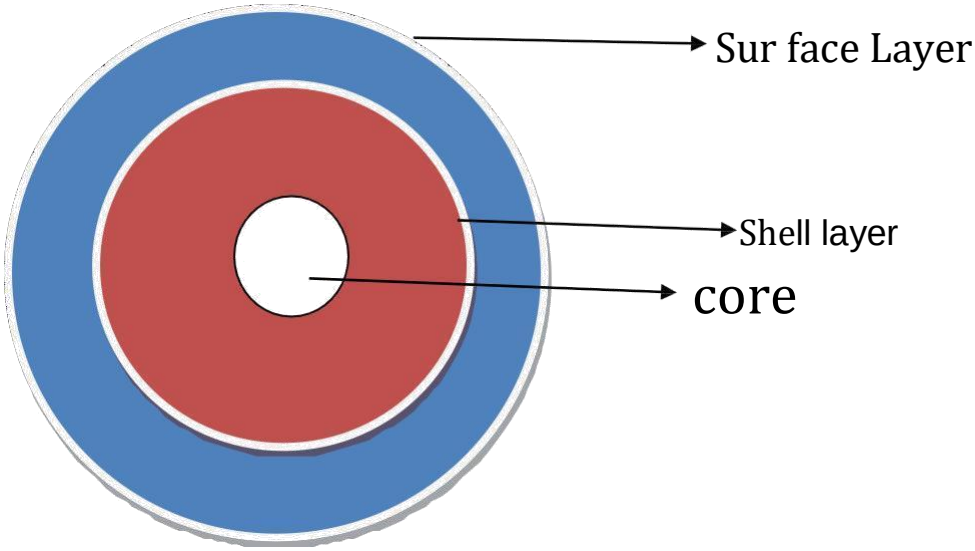
History and development of nanoparticles: -

As far back 4,500yrs ago, humans already exploited the use of nanowires in ceramic matrixes (Dasgupta et al., 2015).The ancient Egyptians were also using nanomaterial's more than 4000yrs ago to synthesize 5nm diameter PbS NPs for hair dye.(Walter et al., 2006). Similarly, "Egyptian blue" was the first synthetic pigment which was prepared and used by Egyptians in nanometer sized glass and quartz around 3rd century BC(Johnson-McDaniel et al., 2013). The chemical synthesis of metallic nanoparticles dates back to 14th and 13th century BC, when Egyptians and Mesopotamians started making glass using metals and it lead to the beginning of metallic nanoparticles (Schaming D,Remita H. Found Chem. 2015).From the late Bronze age(1200- 1000BC), red glass has been found in Italy that is colored by SPR excitation of Cu NPs(Artioli G, Angelini I, Polla A. Phase Transitions. 2008). A roman glass work piece is one of the best example of use of metallic nanoparticles. The Lycurgus cups are a 4th century Roman glass cup, made up of dichroic glass that displays different colors: red when a light passes from behind and green when a light passes from the front (Leonhard U. Nat Photonics. 2007).Recent studies found that the Lycurgus cups contain Ag-Au alloy NPs, with a ratio of 7:3 in addition to about 10%Cu(Freestone I, Meeks N, Sax M, Higgitt C. Gold Bull. 2007).Later, red and yellow colored stained glass found in medieval period churches which were produced by incorporating colloidal Au and Ag NPs respectively (Schaming D, Remita H. Found Chem. 2015).

Nanoparticle structure:-

A nanoparticle is composed of three layers ;

- surface layer (i.e, functionalized and made up of metal ions ,surfectants and polymers)
- shell layer (chemically different from the core)
- core(NPs itself



Structure of nanoparticle

Surface layer:- The surface of nanoparticle is functionalized and made up of metal ions, small molecules, surfactants and polymers.(Park et al., 2002).

Shell layer:- This layer is chemically different from the core. A shell is usually called as a second layer which is chemically different from the core material. Some examples of these materials are core/shell quantum dots which is having a core of one material such as cadmium selenide and the shell of another i.e zinc sulphide (Malik et al., 2002).

The core:- This is the center of nanoparticle or nanoparticle itself.(Wuisteret al., 2004). The core of nanomaterial plays important role in nanoparticle toxicity, but it doesn't mean that the environmental behavior of NPs will be dominated by core constituents. Another important consideration when we discuss the core of nanoparticle is that nanoparticle may be prepared in a pure single phase. In some case, two phases are present and it is assuming that the immense behaviour of nanoparticle are independent to the phase (Rempel et al.,2006).

Properties of nanoparticles:-

The use of nanoparticles is dependent on factors such as size and shape. The nanoparticles are produced at the Nano scale and examine, quantify and manipulative. The chemical structure, size, shape and appearance of nanoparticle are dealt within the synthesis process. Because of their vast surface area, nanoparticles have unique size dependent characteristics .Nanoparticles physical properties differ from bulk materials, allowing it for novel uses. NPs' physicochemical properties, such as their enormous surface area, mechanical strength, optical activity, and chemical reactivity, distinguish them and make them suitable for a wide range of applications in science and technology.

Electronic and optical properties:-

The optical and electronic properties of NPs are linked. NPs have a strong UV-Vis affinity when compared to bulk material due to local surface Plasmon resonance (LSPR). When conduction electrons are present, they produce a distinctive optical experience (Zare et al., 2013). Depending on size and form, NPs' visual characteristics range from visible to

near- infrared. In the UV–Vis–NIR range, colloidal NPs' optical properties are determined by localized surface Plasmon resonance (Lu et al., 2017). SPR is caused by electromagnetic radiation oscillating conduction electron. Electromagnetic radiation cause Plasmon resonance in free electron on NPs. A resonance occurs when incident light photons match the oscillating free electron frequency. For e.g.; Au and Ag NPs have unique bright light due to free electron and SPR band can be seen using a spectrophotometer. The NPs shape and size also influence the color reflected in the spectrum. Small changes inthe NPs form can generate dramatic color variation (Messarahet al., 2012) (Alghuthaymi et al., 2015).

Magnetic properties:-

The literature reveals that the best NPs range from 10-20nm in size and possess un even electron distribution which gives better application value (Zare et al., 2013). Super paramagnetism is a kind of magnetism found in tiny ferromagnetic NPs. The temperature changes the direction of magnetism in these super paramagnetic NPs (Khot et al., 2012)(Jeevanandam et al., 2018).

Mechanical properties:-

Improving surface quality and material removal requires better control of NP mechanical characteristics and interactions with surfaces. Elastic modulus, hardness, movement law, friction, and other mechanical properties of NPs are size-Interfacial adhesion is a term that refers to the adherence of two surfaces together. Tribology makes use of mechanical properties. Nanofabrication, nano manufacturing, surface engineering, and construction/painting materials(Jeevanandam et al., 2018)(Khan et al., 2019).

Thermal properties:-

The thermal conductivity of metal NPs is higher than that of solid fluids. Traditional heat transfer fluids and fluids containing micro particles are expected to outperform nano fluids. High- surface-area particles are desirable because heat transfer happens at the particle surface. It has uses in nano fluids and stable suspensions. Nanotechnology studies electrical, optical, and magnetic activity at the molecular and sub-molecular level(El-Bayoumy et al., 2015).

Classification of nanoparticles:-

An elaborate description of these nanoparticles is provided in the following section: -
Nanoparticles based on origin- On the basis of origin NPs are broadly
classified in to two; natural and synthetic.

Natural nanomaterials: -

These are present in hydrosphere, atmosphere, lithosphere and biosphere. The earth is surrounded by various nanoparticles in the form of rocks, soils, magma or lava in the biosphere. These naturally occurring nanomaterials are produced in two ways; i.e;

through biological species and through anthropogenic activities. These type of nano-particles are found in the form of volcanic ash, fine sand and dust, ocean spray, and even biological matter (for eg- viruses).

Synthetic(engineered) nanomaterials:-

These types of nanomaterials are synthesized mainly by four methods, they are: physical, chemical biological or hybrid methods. Some examples of synthetically engineered nanoparticles with metal oxides such as zinc oxide and titanium dioxide are used in paints, varnish and cosmetics. Zircon and aluminum oxide nano powders are used as components in ceramics to improve both hardness and breaking strength.

Nanoparticles based on dimensional structures-

On the basis of dimension, nanoparticles are of two types: one dimensional two dimensional, three dimensional.

One-dimensional nanoparticles:-

As one-dimensional nanoparticles have thin film or manufactured surfaces they are widely used in electronics, chemistry and engineering since last few decades. The size of the film ranges between 1 and 100nm and are used as monolayer for the production of solar cells or in catalysis. Also the film having wide range of applications in the field of chemical sensors, biological biosensors, memory and storage, magneto-optics, optical devices and fiber optic systems.

Two-dimensional nanoparticles:-

Two dimensional nanoparticles are also known as carbon nanotubes (CNTs). These are composed of hexagonal network of carbon atoms i.e; 100nm in length and 1nm in diameter and these tubes are surrounded by a layer of graphite and appear in the form of cylinder. These nanotubes are capable of carrying high current density, up to 1 billion m^2 making them a superconductor. The mechanical strength of CNTs is 60 times greater than that of steel, it results having greater molecular absorption capabilities and chemical stability.

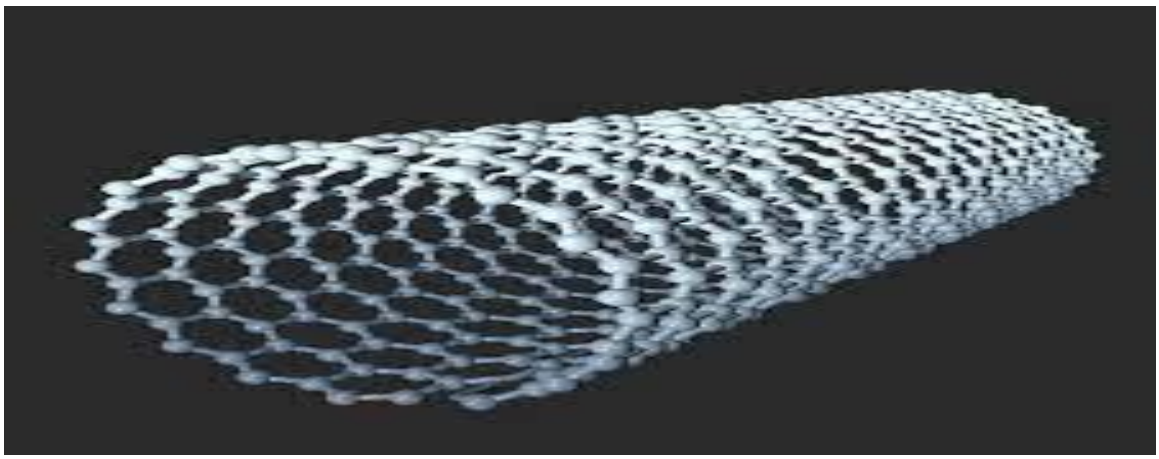


Fig- Carbon nanotubes(CNTs) Ren, G. (2018, November 16)

Three-dimensional nanoparticles:-

They can carry more than two-dimensional structures, based on which these are classified in to three types:

-Fullerenes(carbon60),-Dendrimers,-Quantum dots.

-Fullerenes(C60):- these are spherical cage like structures with the core central atom C60 surrounded by 28-100 carbon atoms. The whole configuration looks like a soccer or hollow ball composed of interconnected carbon pentagons and hexagons. They have unique properties , as they can withstand extreme pressure and retain their original shape. These are used as a lubricants as the molecules present in them are not combined with each other and thus gives them a major support. They are used in the production of electronics as they have distinguishing electrical properties and also having a wider range of applications in the production of biologically active molecules, medical applications and solar cells.

-**Dendrimers:-** these are a novel form of polymers with nanometric dimensions which facilitates in drug delivery and imaging. Size ranges from 10-100nm which have been used in determination of functional groups and thus determining their ideal behavior. On a wider scale, they have been used in diagnostics as they have specialized encapsulated functional molecules. These are having wide range of applications in pharmaceutical industry for the production of some compounds such as non-steroidal anti-inflammatory formulations, antiviral drugs, antimicrobial formulations, anticancer drugs, prod rugs and screening agents for drug discovery.-Quantum dots:- these are small devices having a set of electrons with colloidal semiconductor ranging from 2-10 nm. The variation in electrons from 1 to 1000, and the size, shape and no. of electrons should be monitored. Production of quantum is mainly by two methods; colloidal synthesis and electrochemistry. These are widely used in production of semiconductors, metals, insulators, magnetic materials, metallic oxides, optoelectronic devices ,quantum computing and information storage. Some color coded quantum dots are present which is used in fast DNA testing. Provides wider surface area for the attachment of therapeutic agents, used for targeted drug delivery, bio imaging and tissue engineering. Some examples of quantum dots are: indium arsenide, cadmium telluride, indium phosphide and cadmium selenide.

Nanoparticles based on material:-

On the basis of material NPs are classified in to four types:-

carbon- based nanomaterials

Inorganic nanomaterials

organic nanomaterials

composite-based nanomaterials.

Carbon-based nanomaterials:-

Carbon nanotubes (CNTs) and fullerenes are two primary groups of carbon-based NPs. Nanomaterials comprised of globular hollow cages, such as allotropic carbon forms, are found in fullerenes. Electrical conductivity, high strength, structure, electron affinity, and adaptability have all sparked commercial interest in them (Astefanei et al., 2015). These nanomaterials are formed by two methods: arc discharge and chemical vapour deposition. Chemical vapor deposition is used to formulate all types of carbon derivatives, except carbon8.

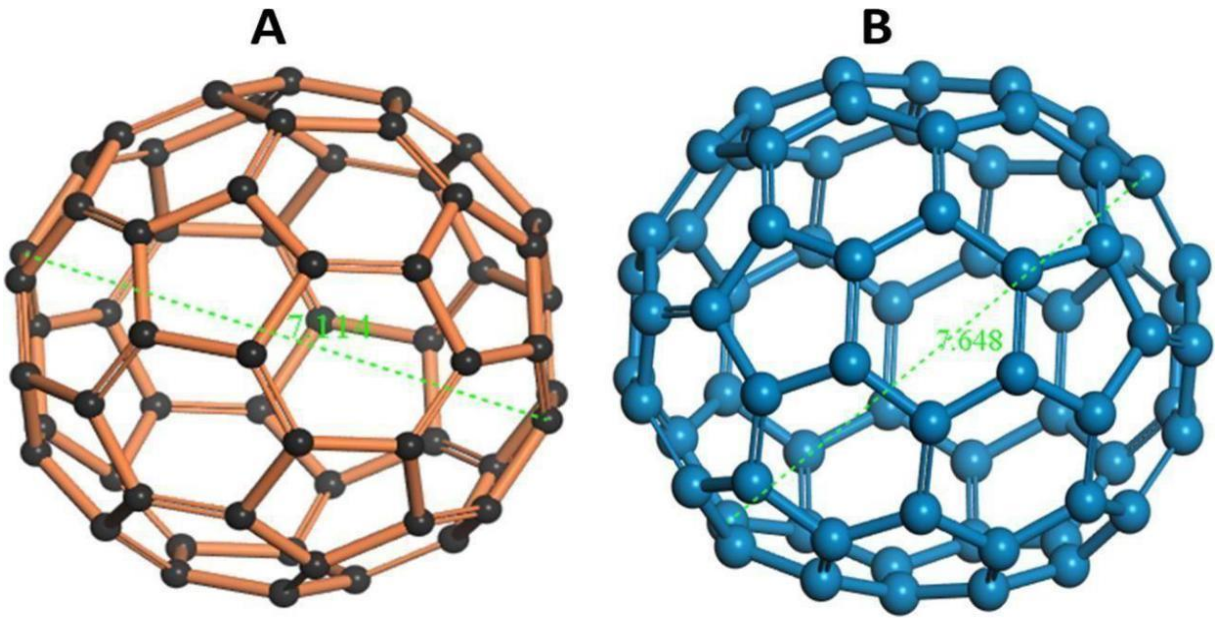


Fig: different form of fullerenes(a)C60,(b)C70(Khan et al., 2019).

Inorganic nanomaterials:- these nanomaterials are not made up of carbon. These includes metals and metal oxides. These NMsare used in synthesis of silver and gold NPs, metal oxides such as titanium oxide and zinc oxide, and semiconductor NPs such as silicon and ceramics.-Ceramic NPs: inorganic non-metallic solids are used for the composition of ceramics. These are synthesized by heat and successive cooling. Their morphology is found to be amorphous,polycrystalline, dense, porous, or hollow. Thus, they have wide range of applications in catalysis, photocatalysis, photodegradation of dyes, and imaging.

Organic nanomaterials:-Organic nanoparticles or polymers are generally known as dendrimers, micelles, liposomes, and ferritin, among others. These nanoparticles are biodegradable and non-toxic, and some, like micelles and liposomes, have a hollow core also known as nanocapsules, which are sensitive to thermal and electromagnetic

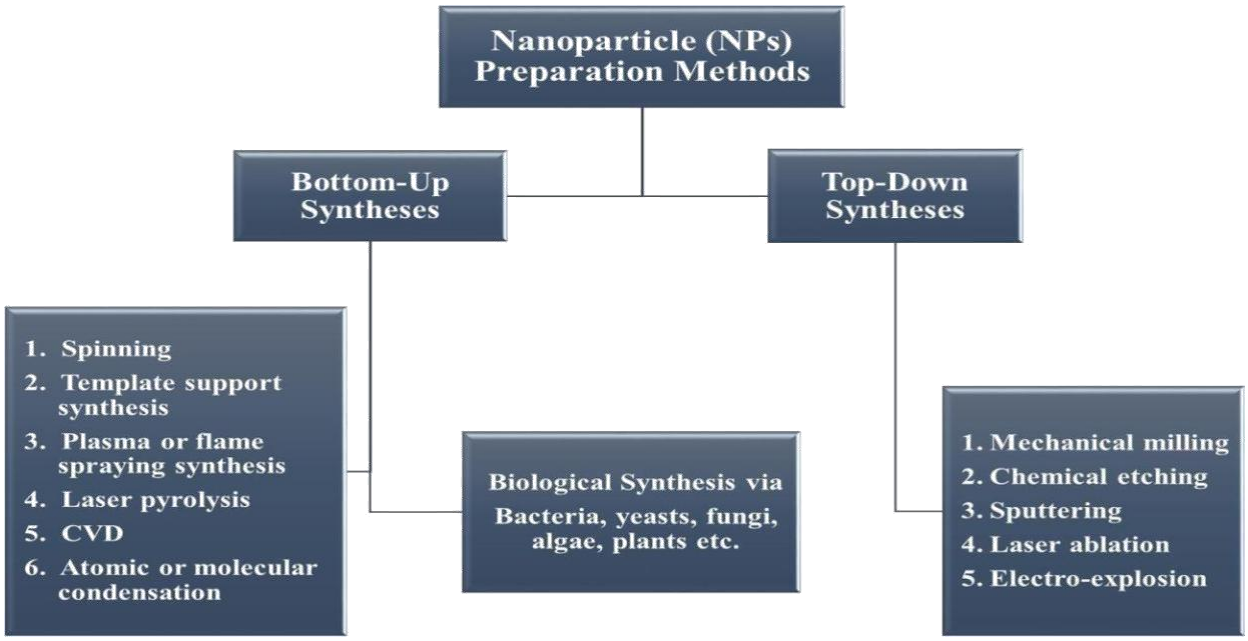
radiation(Tiwari et al., 2008). Because of their distinct properties,they are an excellent alternative for drug delivery. The drug's carrying capacity, stability, and delivery methods, whether entrapped or adsorbed, impact the use and efficiency of the drug. Aside from their usual properties like size, composition, surface shape, and so on, The Organic nanoparticles are commonly employed in biological applications, such as medication delivery systems. Because they are effective and may be injected into particular regions of the body, they are sometimes referred to as drug distribution with precision.Because they are effective and may be injected into particular regions of the body, they are sometimes referred to as drug distribution with precision.-Polymeric NPs: these are organic NPs in which a special polymer is used to encapsulate the nanoparticle, which leads to the formation of polymeric NPs. These NPs are easily configured and have lipid moieties, which leads to their use in biomedical applications. Surfactants and emulsifiers are used to stabilize the external core of nano lipid polymers, which helps in designing and synthesizing these NPs. These are used in the field of drug delivery and RNA synthesis in cancer therapy.

Composite-based nanomaterials:-

These are monophasic in nature. This consist of combinations ofcarbon-based, metal-based, or organic-based.-Semiconductor NPs: Semiconductor materials have qualities that are intermediate between metals and nonmetals, and as a result, they have a wide range of applications in the literature(Ali et al., 2017)(Khan et al., 2017). Because semiconductor NPs have large band gaps, band gap tuning caused considerable changes in their characteristics. As a result, they are vital components in photocatalysis, photo optics, and electronic devices (Shouheng et al., 2000).

Synthesis of nanoparticles:-

For the synthesis of NPs, a variety of strategies can be used, although they are generally categorized into two categories: (1) bottom-up approaches and (2) top-down approaches.(Wang & Xia, 2004).as shown in scheme1(Iravani, 2011).These methods are further divided into subcategories based on the operation, reaction state, and procedures used.



Scheme 1 : Typical synthetic methods for NPs for the ▻top-down and ▻bottom-up approaches. Biosynthesized nanoparticles have been used in a variety of applications including drug carriers for targeted drug delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, and magnetic resonance imaging(MRI).

Green synthesis:-

AuNPs have been synthesized using a variety of procedures, the majority of which are traditional chemical methods. However, there are some significant limitations to these technologies in the form of harmful chemicals that are utilized during production or later in their use. Because of environmental concerns, the toxic reducing agent utilized in the production of AuNPs is difficult to dispose of. As a result, some processes require higher temperatures, which generate a lot of heat and can result in the production of very expensive AuNPs. Many researchers have been drawn to the environmentally friendly approaches utilized to make AuNPs. This is due to the cost- effective, quick, and non-infirm nature of producing in a single step at normal air pressure and ambient temperature.

Characteristics of gold nanoparticles:-

- Gold nanoparticles are chemically inert.
- Having greater biological compatibility.
- Au NPs exhibit optical properties like SPR(Surface Plasmon Resonance).
- Due to their ready functionalization through thiol linkage, these exhibit versatility.

- Gold nanoparticles accumulate in the cancerous cell and show the cytotoxic effect such as apoptosis or necrosis of the specific cell and specific cell receptor.
- Having high stability due to gold sulphur bonds.
- For the drug release to remote place, their photo physical properties can be exhibited.

Advantages of nanoparticles:-

Nanoparticles have two primary basic features that make them advantageous for delivery of drug. First, nanoparticles may pass through smaller capillaries and be taken up by cells, allowing for efficient drug accumulation at the target areas due to their small size. The use of biodegradable materials as a second option. The use of nanoparticles allows for long-term medication release within the target region, lasting days or even weeks (Shinde et al., 2012). There are a few more advantages to nanoparticles in terms of manufacturing and drug delivery. Nanoparticles are quite simple to make, which is why they are used in drugs to target specific areas. Nanoparticles penetrate microscopic capillaries and are taken up by cells, allowing for effective medication accumulation at target areas throughout the body. Nanoparticles in drug delivery provide excellent size control and protection for the encapsulated substance. The clearance time of a medication that is retained at the active site is longer. Improved therapeutic efficacy while also increasing bioavailability. They improved drug stability by reducing fed-to-fasted variability. Stable dosage forms of drugs that are either unstable or have unacceptably low bioavailability in stable dosage forms(Shinde et al., 2012)(Yadav et al., 2013).

Disadvantages of nanoparticles:-

When discussing the benefits and drawbacks of nanotechnology, we must also consider what could be considered the technology's drawbacks. Nanotechnology has also increased the risk to human health; nanoparticles, due to their small size, can cause respiratory problems and a variety of other lethal diseases by simply inhaling nanoparticle-contaminated air for 60 seconds. Nanotechnology is now exceedingly expensive, and creating it can be very costly. It's also harder to create, which is partly why nanotechnology-based products are more expensive (Nanogloss, 2015,08). Nanotechnology has improved people's living standards, but it has also increased pollution, such as water pollution and air pollution. Nano pollution is the term for pollution caused by nanotechnology. This type of pollution is extremely harmful to living things. The drawbacks of nanoparticles are mostly unexplored. As a result, there are just a few more dependent on drug delivery. Because nanoparticles have limited targeting abilities, it is not possible to discontinue therapy. The use of nanoparticles for

drug delivery results in cytotoxicity and alveolar inflammation. The disruption of autonomic balance caused by nanoparticles has a direct impact on heart and vascular function. Nanoparticles exhibit particle growth, erratic gelation, erratic polymeric transmission dynamics, and, on occasion, burst release(Shinde et al., 2012)(Yadav et al., 2013).

Characterization of nanoparticle:-

Characterization of nanoparticles can be done by various techniques such as electron microscopy(TEM, SEM), SAED, atomic force microscopy(AFM), dynamic light scattering(DLS), x-ray photoelectron spectroscopy(XPS), powder x-ray diffraction(XRD), Fourier transform infrared spectroscopy(FTIR), ultraviolet-visible spectroscopy(UV-VIS), dual polarization interferometry and nuclear magnetic resonance(NMR). Some are explained below:

UV-visible absorption spectroscopy:-

This technique is used to determine the optical properties of a solution. A light is send through the sample solution and the amount of light that is absorbed is measured. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law (Subbaiya, R.,Shiymala,M.,et.,2014). UV-visible spectrography in the region of 216-265nm revealed a suitable SPR with high band intensities and peak (Monalisa Pattanayak and P.L 2013).

X-ray diffraction (XRD) analysis:-

This is a conventional technique for determination of crystallographic structure and morphology. This technique is used to establish the metallic nature of particles and also provide information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell.

Fourier transform infrared(FTIR) spectroscopy:-

This technique helps in determining the nature of associated functional groups and structural features of biological extracts with nanoparticles. It measures infrared intensity vs. Wavelength of light. The calculated spectra clearly reflect the well-known dependence of NPs optical properties. By using FTIR, the synthesis of silver NPs by various plant extract was analyzed and showed characteristic peak (Amudha Murugan, Krishna Kumara, Shanmugasundaram, 2014).

Microscopic techniques:-

These techniques include transmission electron microscopy (TEM) and scanning electron microscopy(SEM), helps for the study of morphology of nanoparticles. Many

researchers used these techniques to show that the synthesized NPs were more or less uniform in size and shape (Shobha, G., Vinutha Moses, Ananda, and S. 2014).

Transmission electron microscopy (TEM):-

Transmission electron microscopy (TEM) is a high magnification measurement technique that depicts the passage of an electron beam through a sample. The amplitude and phase variations in the transmitted beam provide imaging contrast that varies with sample thickness (the amount of material that the electron beam must pass through) and sample material (heavier atoms scatter more electrons and therefore have a smaller electron mean free path than lighter atoms). TEM imaging has significantly higher resolution than light-based imaging techniques because it illuminates the sample with electrons rather than light. The preferred method for directly measuring the particle size, grain size, size distribution, and morphology of nanoparticles is TEM imaging. Sizing precision is typically within 3% of the actual value.

Scanning electron microscopy (SEM):-

It helps in the determination of size, shape and morphologies of formed nanoparticle. SEM gives high resolution images of the surface of the sample. It works on the principle of optical microscope, but the difference is that it measures the electrons scattered from the sample instead of photon. SEM is capable of magnifying images up to 200,000 times (Umer et al., 2012).

Dynamic light scattering(DLS):-

DLS (Dynamic Light Scattering) is a useful technique for characterizing nanoparticles and other colloidal solutions. DLS measures the amount of light scattered by a laser as it passes through a colloidal solution. The size of the particle in solution can be determined by analysing the modulation of the scattered light intensity as a function of time. An autocorrelation function based on DLS. The nanoparticle diffusion rate is represented bythe time delay at which the function decreases. The analysis is based on the diffusive motion of particles in solution (Brownian motion), which states that larger particles move slower and scatter more light than smaller particles.

Zeta potential:-

The zeta potential (also known as the electro kinetic potential) quantifies the charge stability of colloidal nanoparticles by measuring the "effective" electric charge on the nanoparticle surface. When a nanoparticle has a net surface charge, the charge is "screened" by a higher concentration of ions with opposite charges near the nanoparticle surface. This layer of oppositely charged ions moves with the nanoparticle, and the layer of surface charge and oppositely charged ions is known

collectively as the electrical double layer. The Zeta Potential is a measurement of the potential difference between the bulk fluid in which a particle is dispersed and the layer of fluid containing oppositely charged ions associated with the nanoparticle surface. Particles with a positive Zeta potential. The magnitude of the Zeta Potential indicates particle stability, with larger magnitude potentials exhibiting increased electrostatic repulsion and thus increased stability.

Applications of nanoparticles:-

Considering the unique properties, NPs can be used in avariety of applications, some are given below:

Applications in drug and medications:-

NPs have piqued the interest of every branch of medicine due to their ability to deliver drugs at the optimal dosage range, often resulting in increased therapeutic efficiency, reduced side effects, and improved patient complication rates.(Alexis et al., 2008).

Application in manufacturing and materials:-

Nano crystalline materials are very interesting materials for material science because their properties deviate from the properties of the respective bulk material in a size dependent manner. Manufacturing NPs exhibit physicochemical properties that induce unique electrical, mechanical, optical, and imaging properties that are highly sought after in certain applications in the medical, commercial, and environmental sectors(Dong et al.,2014)(Ma, 2003) (Todescato et al., 2016).

Applications of nanoparticles in food:-

Nano food is a term used to describe foods that use nanotechnology techniques, tools or manufactured nanomaterial that have been added during cultivation, production, processing or packaging. There are various purposes for production of nanofood as they helps in improvement of food safety, enhancement of nutrition and flavor, and cutting production and consumer costs. The application of food in nanotechnology is emerging very rapidly and involves all areas of the food chain from agricultural applications to food processing and enhancing bioavailability of nutrients.

Applications of nanoparticles in gene delivery:-

It is a technique that plays important role in introducing a gene of interest in order to express its encoded protein in a suitable host.

Application of nanoparticle in cancer treatment:-

A variety of nanoparticle systems are currently being researched for use in biomedical applications, with a focus on cancer therapeutics. As a result, some precious metals (primarily gold and silver systems, Au and Ag) and magnetic oxides (particularly magnetite Fe3O4), as well as quantum dots and some "natural nanoparticles," have received a lot of attention (Bououdina, M.S.,Rashdan, et al,. 2013).

What are Gold Nanoparticles

A gold nanoparticles (AuNPs) are small gold nanoparticles with the diameter of 1-100nm which, one is dispersed in water, are also known as colloidal gold .A significant attribute of gold nanoparticle is their ability against oxidation and degradation in-vivo. These attractive properties play a considerable role in the advancement of nanomaterials for use as clinical therapeutics and diagnostic tools

Why we use gold nanoparticles

They have suitable properties for control drug delivery, cancer treatment, biomedical imaging, diagnosis and many others, due to their gold nanoparticles are chemically inert. Gold nanoparticles have excellent compatibility with human body, low toxicity and tunable and stability compact dimensions, and the capacity to interact with the range of chemical.

- They have greater biological compatibility.
- Optical property like local surface Plasmon resonance (LSPR) are exhibitant.

On the basis of a forementioned explanation and to enhance the antibacterial efficacy of *kinnow mandarian* peel extract we decided to synthesize gold nanoparticles (K-AuNPs) in a single step process and also evaluated its antibacterial potential in contrast to pure *kinnow mandarian* extract.

OBJECTIVES

Objectives

- ✓ Collection & preparation *Kinnow mandarin* aqueous extract.
- ✓ Synthesis of gold nanoparticles with aqueous extract of *Kinnow mandarin* .
- ✓ Characterization of synthesized gold nanoparticles by UV-visible spectroscopy, DLS, TEM, Zeta potential.
- ✓ Antibacterial activity analysis of pure *K.Mandarin* extract, GNPs in contrast to antibiotic Levofloxacin.
- ✓ Determination of MIC values from MIC graph.

MATERIAL AND METHODS

Plant Collection and Extract Preparation

Fresh peels of *Kinnow mandarin* was collected from local fruit market of Lucknow, India, in March 2022.The peels of the *Kinnow mandarin* rinsed repeatedly with distilled water to remove any impurities from their surface. After it, peels were cut into small pieces and air-dried. The peels were crushed for 2 hours at 4°C using mortar and pestle in the presence of PBS (Phosphate-buffered saline) pH 7.4. Afterward, the paste of peel was centrifuged at 6000rpm for 12 minutes at 4°C. Finally, the supernatant was transferred into 50ml of falcon tube and the pellets were discarded.

***Kinnow mandarin* Mediated single step Synthesis of Gold Nanoparticles**

The *Kinnow mandarin* peel extract was transferred in four different Eppendorf tubes labeled with ZK1, ZK2, ZK3 and ZK4.

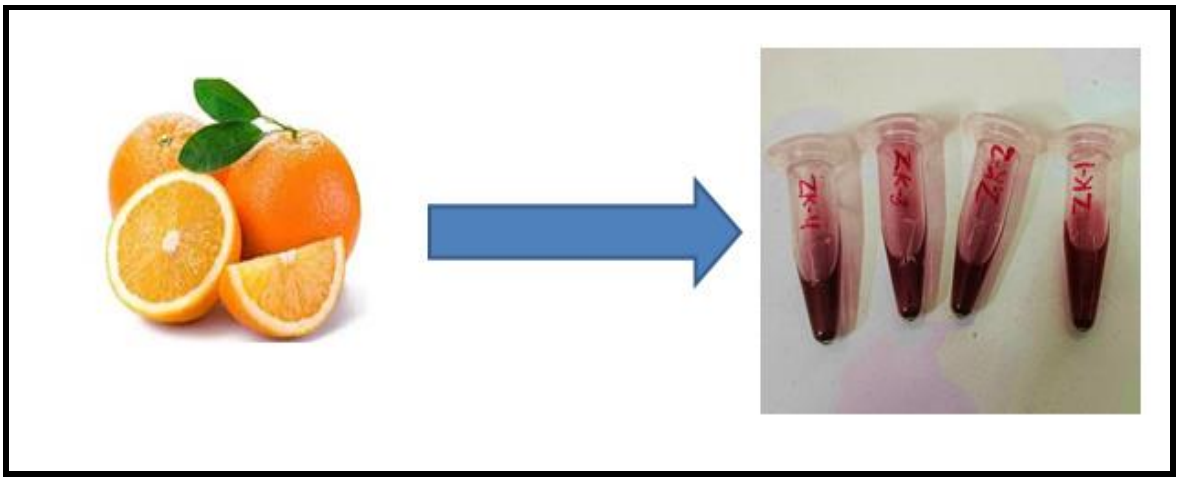
25µl of extract, 10µl of AuCl4 (1mM) and 465µl of PBS was added in ZK1.

50µl of extract, 10µ of AuCl4 (1mM) and 440µl of PBS was added in ZK2.

75µl of extract, 10µl of AuCl4 (1mM) and 415µl of PBS was added in ZK3.

100µl of extract, 10µl of AuCl4 (1mM) and 390µl of PBS was added in ZK4.

PBS was used to maintain the volume up to 500µl. The Eppendorf tubes were incubated at 40°C for 24-72 hours. The transformation of orange color of the solution to ruby red indicated the formation of gold nanoparticles. The gold nanoparticles emulsion obtained was kept at room temperature to check the stability of synthesized Au-NPs & UV-Visible spectroscopy in the range of 250- 800 nm was performed to examine the SPR band of the synthesized nanoparticles.



Characterization of Au-NPs

The transformation of gold salts into gold nanoparticles was confirmed by using UV-vis spectroscopy at regular intervals in the range of 200nm to 800nm (Shimadzu UV-1601 dual-beam spectrophotometer). The method is based on the color shift that occurs when metal salts are reduced to synthetic AuNPs. On carbon-coated copper TEM grids, a single drop of an AuNPs solution was applied. It was then examined using a Tecnai G2 Spirit transmission electron microscope with a BioTwinlens setup from Hillsboro, Oregon. The system is powered by an accelerating voltage of 80 kV80 kV. The hydrodynamic radius of the nanoparticles created was then determined using dynamic light scattering (DLS). The particle size and zeta potential were also determined using a Zetasizer Nano-ZS (ZEN3600 Malvern Instrument Ltd., Malvern, United Kingdom). Transmission electron microscopy was also used to measure the size and form of gold nanoparticles.

Determination of Antibacterial Activity by Disc Diffusion Method

The disc diffusion method was used to determine the antibacterial properties of *Kinnow mandarin* synthesized Au-NPs. For antibacterial assay analysis, pure cultures of *Escherichia coli, Salmonella abony* and *Bacillus subtilis* were obtained from American Type Culture Collection.The suspensions of the strains tested were standardized to 0.5 McFarland. For the study, the suspension was collected from overnight trypticase soy broth (TSB) cultures and spread on Mueller–Hinton (M.H.) agar. During the experiment, four wells were made on the incubated petri-plates and 100µl concentrations of K-AuNPs were added in each well of three incubated petri-plates. Afterward, 20µl of Levofloxacin (1mg/mL) and 200µl concentration of peel extract was added in different wells of each petri-plate. Out of four wells, one well was acting as controller. The agar plates were incubated overnight at 37°C. Following that, the diameter of inhibitory zone was determined.

Evaluation of Minimal Inhibitory Concentration via micro dilution method

The experiment was conducted on a sterile 96-well plate to measure the antibacterial properties and determine the MIC of pure *Kinnow mandarin* aqueous peel extract, K-AuNPs, and Levofloxacin (used as a control), as stated in several papers. In nourishing broth, the crude *Kinnow mandarin* aqueous peel extract and K-AuNPs were serially diluted. After that, each well of 96-well plates was incubated for 24 hours at 37 º C with a standardized bacteria solution (10^3 cells/mL). After 24 hours, the plate was read at nm

using an ELISA reader [(Microplate Reader (BIORAD-680)], and the reading was saved for future use.

The antibacterial findings of this study and MIC readings showed that the encapsulation of *Kinnow mandarin* on AuNPs greatly boosted its effectiveness. K-AuNPs were shown to inhibit the growth of a number of Gram-negative and Gram-positive bacterial strains at much lower concentrations. The antibacterial properties of the AuNPs and the *Kinnow mandarin* may have a synergistic effect, which might clarify why the *Kinnow mandarin* 's antibacterial activity increased following encapsulation over AuNPs. AuNPs work against bacteria largely through two mechanisms: first, they collapse the membrane potential, which reduces ATPase activity and hence the ATP level; second, they interfere with the ribosome's component binding to tRNA. However, unlike many bactericidal medicines and nanomaterials, AuNPs do not contain ROS-related pathways.

RESULTS

Kinnow mandarin Extract Mediated synthesis of AuNPs (K-AuNPs)

This study used *Kinnow mandarin* peel extract as a reducing and stabilizing agent, whereas 1 mM Chloroauric acid (HAuCl4) served as the gold precursor. The synthesis of K-AuNPs is considered to be induced by the aqueous extract's reducing enzymes and capping agents, such as secondary metabolites, which combine to convert AuCl4 (+3 oxidation state) to Au (0 oxidation state). The creation of K-AuNPs was confirmed visually by a shift in the color of the extract from orange to ruby red, indicating gold reduction [33].

Characterization of K-AuNPs

Due to Surface Plasmon resonance, an unusual phenomenon is seen in noble metal nanoparticles (SPR). This imbues the nanoparticle's surface with the character of powerful electromagnetic fields, resulting in scattering and absorption [34]. Thus, the formation of K-AuNPs was confirmed herein using U.V-vis spectra (Fig 1.2). The absorption peak was observed 527nm, which corresponds to the SPR band of the K-AuNPs. The phytoconstituents in *Kinnow mandarin* peel extract reduced the gold salt (HAuCl4) into AuNPs and encapsulated the AuNPs, preventing the nanoparticles from aggregating and providing stability to the K-AuNPs. The change in color from light yellow to ruby red indicated the successful synthesis of K-AuNPs, and the result of the SPR band confirmed that at 523nm.However, there was no discernible peak for *Kinnow mandarin* peel extract.

The transmission electron microscope (TEM) was used to determine the precise size, shape, and 2-dimensional morphology of K-AuNPs, which was determined to be 18d. nm with spherical shape and monodispersed parameter (Fig 1.1).The technique of dynamic light scattering (DLS) was used to determine the average particle size and profile of the particle size distribution of K-AuNPs. K-AuNPs had an average particle size of 64d. nm and a polydispersity index (PDI) of 0.216, indicating a homogeneous size distribution, as shown in (Fig 1.3 A). The zeta potential of K-AuNPs was also investigated (Fig. 1.3 B). Nanoparticle colloidal stability requires a zeta value of -20 mV in most cases [35].

Furthermore, the zeta potential of the prepared K-AuNPs was found to be -17 mV, indicating the significantly high stability of the nanoparticles. When the aqueous dispersion of K-AuNPs was observed at room temperature, no clumping or accumulation was

observed. This was most likely due to the gold nanoparticles' electrostatic repulsive effects. The nanoparticles are prevented from colliding because of this repulsion.

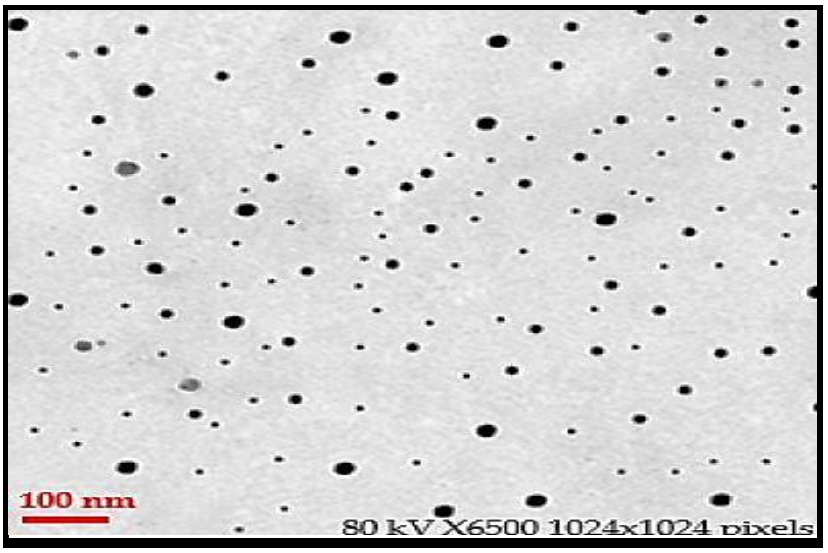


Fig 1.1 TEM micrograph of synthesized K-AuNPs (18 d.nm.)

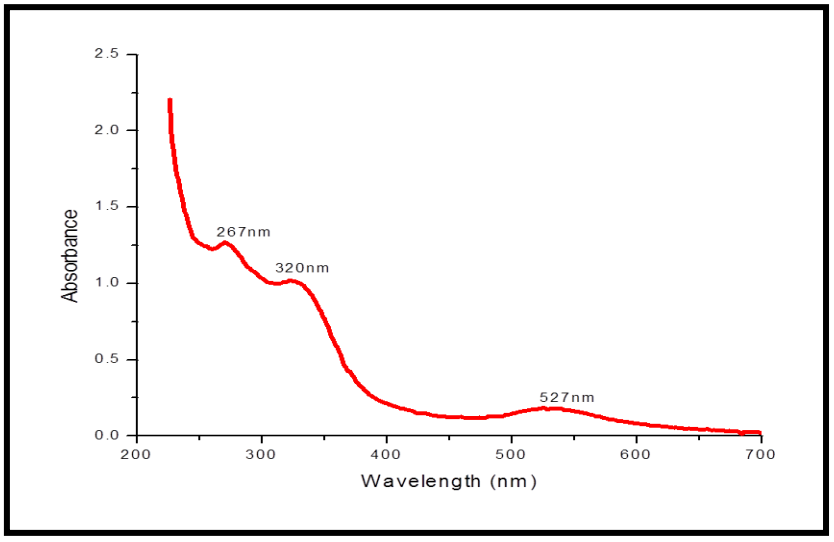


Fig 1.2 UV-Visible spectroscopy of K-AuNPs shows absorption peak (SPR band) of K-AuNPs at 527nm.

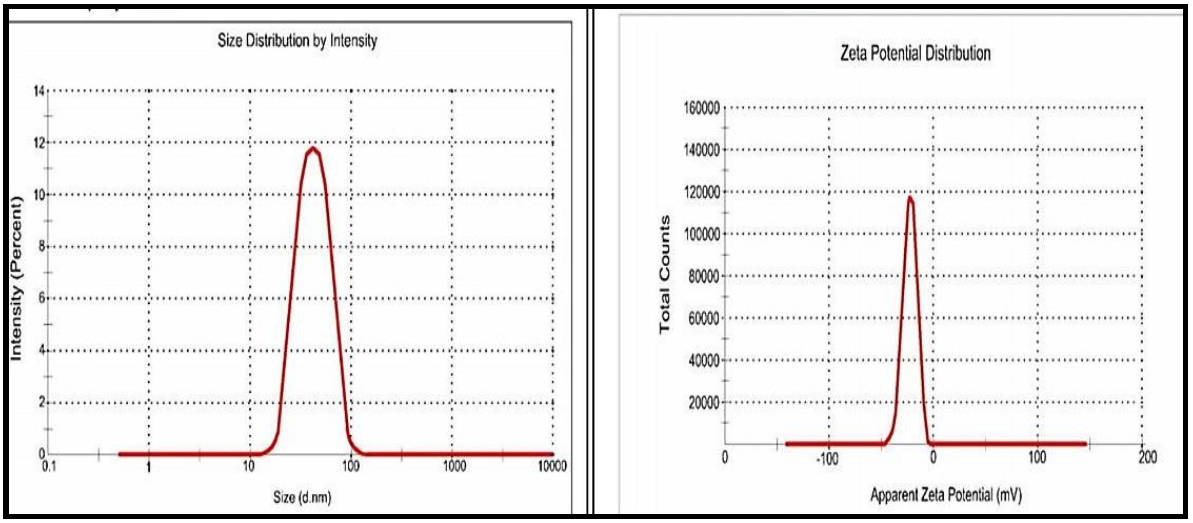


Fig. 1.3 (A) The dynamic light scattering of K-AuNPs (64 d.nm) (including hydrodynamic layer), and (B) The Zeta potential of K-AuNPs (-17mV).

Comparative antibacterial activity of K-AuNPs in contrast to pure *Kinnow mandarin* aqueous extract

Several microbial pathogens are known to cause a variety of infectious diseases. Because of improper usage, these organisms have mutated and developed resistance to a variety of antibiotics. Hence, several researchers are finding alternative system of medicine for the treatment. The present study analyzed the antibacterial potential of crude *Kinnow mandarin* aqueous extract and K-AuNPs against Gram negative (*E.coli*) and Gram positive (*Bacillus subtilis and Salmonella abony*) bacterial strain by the disc diffusion method.

The maximum activity against *B. subtilis, S.abony* and *E.coli* was observed at a concentration 100mg/mL crude aqueous extract of *Kinnow mandarin* , with inhibitory zone of 15±0.2, 11±0.03, and 17±0.9 respectively (TABLE 1). Additionally, in the case of Levofloxacin (1mg/mL) the obtained zone of inhibition was 19±0.2, 16±0.05 and 14±0.1 against *E.coli*, *B.subtilis* and *S.abony*.

However, K-AuNPs have shown a significantly high zone of inhibition at lower concentration (50µg/mL) compared to the crude *Kinnow mandarin* peel extract and Levofloxacin. K-AuNPs established the zone of inhibition 25±0.9, 27±0.1 and 16±0.7 against *B.subtilis, E.coli and S. abony.*

Table 1 Zone of inhibition of *Kinnow mandarian* extract, K-AuNPs and levofloxacin determined against various bacterial strains.

Conc. values	Zone of Inhibition (mm)		
	B. subtilis	S.abony	E.coli
Control	0	0	0
K-AuNPs(50µg/mL)	25±0.9	16±0.7	27±0.1
Extract	15±0.2	11±0.03	17±0.9
Levofloxacin	16±0.05	14±0.1	19±0.2

Evaluation of Minimum Inhibitory Concentration of *Kinnow mandarin* aqueous peel extract and K-AuNPs

The MIC is the minimum concentration of *Kinnow mandarin* aqueous peel extract and K-AuNPs which totally prevents growth of bacteria, whereas the MIC50 is the *Kinnow mandarin* aqueous peel extract and K-AuNPs concentration that inhibits 50% of the microbial community. The MIC50 of *Kinnow mandarin* aqueous peel extract and K-AuNPs were determined against a variety of Gram-negative and Gram-positive bacterial species. *Escherichia coli* had MIC50 value of 2.6µg/mL, 1.1µg/mL, and 2.0 µg/mL (Fig 1.4); *Bacillus subtilis* had MIC50 values of 2.06µg/mL, 1.3µg/mL, and 1.9µg/mL (Fig 1.5); *Salmonella abony*, 2.1µg/mL, 1.5µg/mL, and 1.7µg/mL (Fig 1.6) of pure *Kinnow mandarin* aqueous peel extract, K-AuNPs respectively. The inclusion of phytoconstituents in the plant might explain the high antibacterial activity found. According to recent studies, alkaloids-rich plants have a large level of antibacterial action. Our extracts even include considerable amounts of flavonoids, tannins, saponins, gums, and mucilages, that might explain its exceptional antibacterial properties[36]. The K-AuNPs are substantially more effective at lower dosages than pure *Kinnow mandarin* aqueous peel extract and Levofloxacin against the aforementioned bacterial pathogens, according to our MIC data. The encapsulation of K-AuNPs with active phytoconstituents in *Kinnow mandarin* yielded in *Kinnow mandarin* abundance reduced, whereas K-AuNPs effectiveness and stability rose. Because AuNPs operate as a carrier for *Kinnow mandarin* , they (AuNPs) also exert a protective effect. By compressing the membrane potential and inhibiting the ATPase, it has antimicrobial properties. The bacterial cell wall's ATP level changes as a result of these processes [37]. The ability of *Kinnow mandarin* and AuNPs to fight bacteria together might be beneficial as they together have boosted antibacterial activity. Prior research has found antibacterial efficacy in aqueous and methanolic extracts of *Kinnow mandarin* seed when tested using the agar well diffusion method against *Escherichia coli,*

Serratia marcecens, Salmonella sp., Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter sp., Proteus mirabilis and Shigella sp. [38].

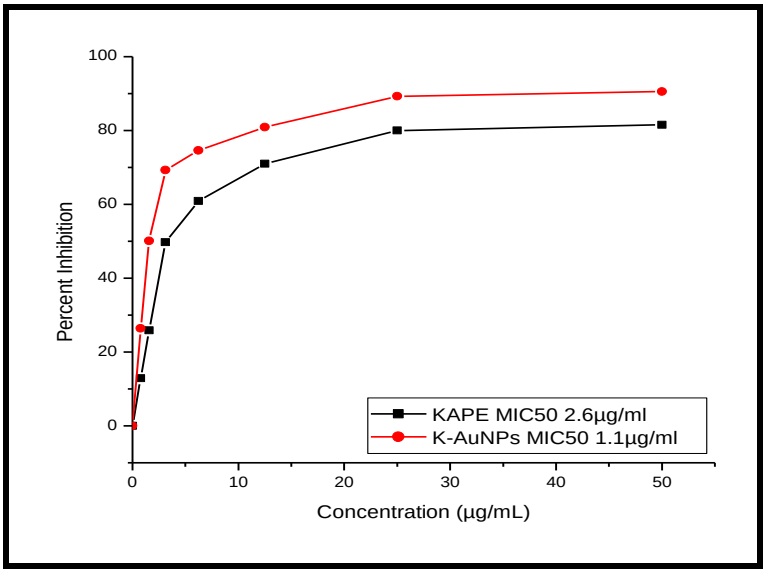


Fig 1.4. Minimum Inhibitory Concentration of pure KAPE, K-AuNPs against *Escherichia coli.*

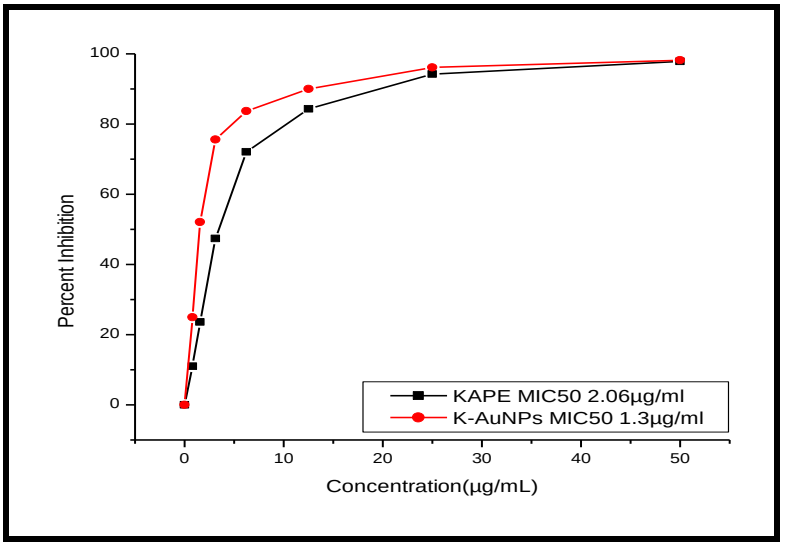


Fig 1.5. Minimum Inhibitory Concentration of pure KAPE, K-AuNPs against, *Bacillus subtilis.*

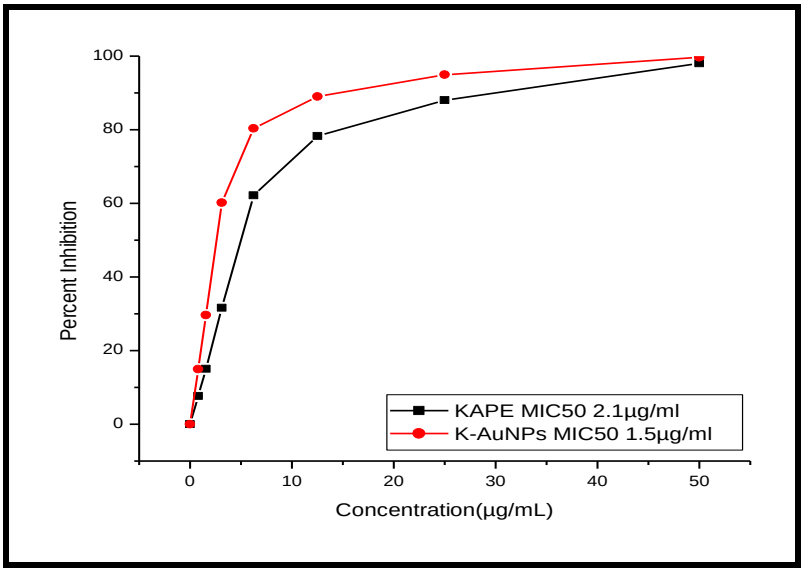


Fig 1.6 Minimum Inhibitory Concentration of pure KAPE and K-AuNPs against, *Salmonella abony.*

CONCLUSION

The current study demonstrates significant sized, monodispersed and spherical gold nanoparticles can be synthesized via a single step process using *Kinnow mandarian* peel extracts. The active phytoconstituents of the plant are itself able to cap the synthesized nanoparticles (K-AuNPs) after its reduction process. The size of the synthesized gold nanoparticle (K-AuNPs) was found to be 18 d. nm as confirmed by TEM and are highly stable in nature (zeta potential ~ -17mV).

The key findings of the performed study demonstrates that K-AuNPs (offered at a lower dose than pure R. serpentina) can substitute for *Kinnow mandarin* due to the increased antibacterial potential. Thus, the study's findings indicate that the crude *Kinnow mandarin* aqueous peel extract and K-AuNPs may effectively treat bacterial infections. However, additional investigations are required to determine the active phytoconstituent of the *Kinnow mandarin* , which might be responsible for reducing gold salt into its nano form and coupling on its surface to stabilize the synthesized K-AuNPs. However, in future more investigations are needed to determine the *in vivo* activity of K-AuNPs before they can be declared suitable drug carriers in the medical domain.

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