

A GREEN APPROACH FOR THE SYNTHESIS OF TITANIUM NANOPARTICLES USING ZINGIBER OFFICINALE ROSCOE AND PHYLLANDHUS EMBLICA EXTRACTS

Submitted in partial fulfillment of the requirements for the award of Bachelor of Science
degree in Biotechnology.

By

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(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A++" by NAAC

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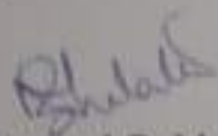
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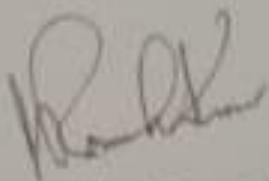
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Sarmila S (41300041), who carried out the project entitled "A GREEN APPROACH FOR THE SYNTHESIS OF TITANIUM NANOPARTICLES USING ZINGIBER OFFICINALE ROSCOE AND PHYLLANDHUS EMBLICA EXTRACTS" under my supervision from October 2023 to April 2024.


Internal Guide

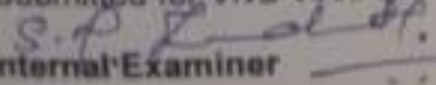
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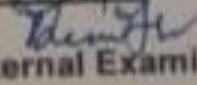

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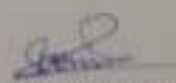

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DECLARATION

I, Sarmila S (41300041) hereby declare that the Project Report entitled "A GREEN APPROACH FOR THE SYNTHESIS OF TITANIUM NANOPARTICLES USING ZINGIBER OFFICINALE ROSCOE AND PHYLLANDHUS EMBLICA EXTRACTS" done by us under the guidance of DR..M.Bavanilatha (internal guide) Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Microbiology.

DATE: 27 Oct 24

PLACE: CHENNAI


SIGNATURE OF THE CANDIDATE

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I convey my thanks to **Dr.Rekha Chakravarti M.E., PHD** Dean, school of science and Humanities And **DR.V. RAMESH KUMAR, M. Phil, Ph.D., Head of the Department, Department of Biotechnology** for providing us with the necessary support and details at the right time during the progressive reviews.

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ABSTRACT

The plant kingdom occupies the predominant niche in the biodynamics of titanium nanoparticles, particularly within medicinal plants, demonstrating substantial potential in biotechnological applications. This investigation focuses on the green synthesis of titanium derived from *Zingiber officinale* Roscoe and *Phyllanthus emblica*, infrared spectroscopy, X-ray diffraction, scanning electron microscopy, and transmission electron microscopy. The synthesized particles were characterized and visible spectroscopy revealed a Plasmon resonance band at 352.4 nm on the titanium surface. The Fourier- transform infrared analysis highlighted discernible interactions between titanium and active microbes in *Zingiber officinale* Roscoe and *Phyllanthus emblica* extracts, which highly influences the synthesis and stabilization of titanium nanoparticles, X- ray diffraction demonstrated the particle's crystalline structure, with a semi-Gaussian profile of 38-49°. Scanning electron microscopy imaging showcased spherical atoms with an average diameter of 17 nm. Transmission electron microscopy displayed spherical and semi-spherical TiO₂NPs with an average size of 43-56 nm. These nanoparticles exhibited potent antimicrobial activity against pathogens, bacteria, and fungi, demonstrated through the 12-well diffusion method. at a concentration of 1000 µg/ml, exhibited inhibition. Against bacteria, this concentration showed inhibitory effects on *Staphylococcus aureus*, *Zingiber officinale* Roscoe, and *Phyllanthus emblica*, Furthermore, TiO₂NPs displayed concentration-dependent cytotoxic effects against human heart cancer cells. The robust activity of green-synthesized titanium nanoparticles from medicinal plants recommends their medicinal use as antimicrobial and cytotoxic agents.

TABLE OF CONTENTS

S.NO NUMBER	LIST OF TOPICS	PAGE
1	Bonafide certificate	1
2	Declaration	2
3	Acknowledgement	3
4	Abstract	4
5	List of tables	5
6	list of figures	6
7	list of abbreviations	7
8	CHAPTER 1- Introduction	
10		
	1. Properties	11
	2. Synthesis	12
	3. Characterization	13
9	CHAPTER 2- Literature survey	14
10	CHAPTER 3 – Aim and scope	16
	1. Aim	17
	2. Aim and scope of the present investigation	19

11	CHAPTER 4 –Materials and methods	21
	1.preparation of fruit extracts of zingiber and amla	23
	2.preparation of 1 mm titanium nitrate solution	25
	3.synthesis of titanium nanoparticles	26
	4.Analysis of synthesized titanium nanoparticles	
using	UV- visible spectrophotometry	28
	5.Sources of UV radiation	30
	6.Sources of visible radiation	31
	7. wavelength selctor or (monochromater)	33
	8. cuvettes	35
	9.Detectors	36
	10.procedure	40
	11.Characterization of synthesized titanium	
	Titanium nanoparticles using SEM and EDX	42
	12.procedure	42
	13.EDX	43
	14.Antimicrobial studies	44
	15.cytotoxicity assay	46
	16.cell line maint enance and growth conditions	47
	17.MTT assay –equipment required	50

12	CHAPTER 5 –Result and discussion	51
	1.synthesis of titanium nanoparticles	52
	2 .Analysis of synthesized titanium nanoparticles	54
	3.characterization of synthesized titanium nanoparticles using sem and edx	54
	3. Antimicrobial studies	55
	4. Cytotoxic effect	56
13	CHAPTER 6 – Summary and conclusion	58
14	References	68

LIST OF ABBREVIATION

TiO ₂	Titanium Nanoparticle
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive X-Ray Spectroscopy
FTIR	Fourier Transmission Infrared Spectroscopy
XRD	X-Ray Diffraction
EDX	Energy Dispersive X-Ray
S. TYPHI	Salmonella Typhi
E. COLI	Escherichia Coli

FIGURE NO	FIGURE NAME
1	Synthesis of titanium Nanoparticle
2	SEM image of titanium nanoparticle at 10.01kx magnification
3	SEM image of titanium nanoparticle at 4.24 kx magnification
4	SEM image of titanium nanoparticle at 10.00 kx magnification
5	SEM image of titanium nanoparticle at 100.58 kx magnification
6	SEM image of titanium nanoparticle at 50.11 kx magnification
7	SEM image of titanium nanoparticle at 100.64kx magnification
8	SEM image of titanium nanoparticle at 25.25 kx magnification.
9	SEM image of titanium nanoparticle at 25.38 kx magnification
10	SEM image of titanium nanoparticle at 25.49 kx magnification
11	EDS analysis
12	EDS electron image
13	EDS electron image
14	<i>Pseudomonas aeruginosa</i>
15	Anticancer test
16	Anticancer test

LIST OF TABLES AND GRAPHS

S.NO	TABLES AND GRAPH NAME
1.1	Table and Graph for SEM& EDS
1.2	Graph for XRD
1.3	Graph for FTIR
1.4	Table of Antibacterial test
1.5	Table of Anticancer test

CHAPTER 1

1.1 INTRODUCTION

Nanotechnology is a natural end-result of scientific development and our ability to understand and manipulate matter at smaller and smaller levels. It is a tremendously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical application on early disease detection, treatment, and prevention.

In [nanotechnology](#), a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. It is further classified according to size: in terms of [diameter](#), fine particles cover a range between 100 and 2500 [nanometers](#), while ultrafine particles, on the other hand, are sized between 1 and 100 nanometers. Similar to ultrafine particles, nanoparticles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of

most molecules would fit into the above outline, individual molecules are usually not referred to as nanoparticles.

[Nanoclusters](#) have at least one dimension between 1 and 10 nanometers and a narrow size distribution. [Nanopowders](#) are agglomerates of ultrafine particles, nanoparticles, or nanoclusters. Nanometer-sized [single crystals](#), or single-domain ultrafine particles, are often referred to as [nanocrystals](#). Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields.

Nanoparticles have a very high surface area to volume ratio, which provides a tremendous driving force for [diffusion](#), especially at elevated temperatures. [Sintering](#) can take place at lower temperatures, over shorter time scales than for larger particles. This theoretically does not affect the density of the final product, though flow difficulties and the tendency of nanoparticles to agglomerate complicates matters. The large surface area to volume ratio also reduces the incipient [melting temperature](#) of nanoparticles.

Moreover nanoparticles have been found to impart some extra properties to various day to day products. For example the presence of titanium dioxide nanoparticles imparts what we call the self-cleaning effect, and the size being nanorange, the particles can not be observed. Zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions.

1.2 Properties

Nanoparticles are effectively a bridge between bulk materials and [atomic](#) or [molecular](#) structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of

atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material.

An excellent example of this is the absorption of solar radiation in photovoltaic cells, which is much higher in materials composed of nanoparticles than it is in thin films of continuous sheets of material. In this case, the smaller the particles, the greater the solar absorption.

Other size-dependent property changes include [quantum confinement](#) in [semiconductor](#) particles, [surface plasmon resonance](#) in some metal particles and [superparamagnetism](#) in [magnetic](#) materials. Ironically, the changes in physical properties are not always desirable. Ferroelectric materials smaller than 10 nm can switch their magnetisation direction using room temperature thermal energy, thus making them unsuitable for memory storage.

[Suspensions](#) of nanoparticles are possible since the interaction of the particle surface with the [solvent](#) is strong enough to overcome [density](#) differences, which otherwise usually result in a material either sinking or floating in a liquid. Nanoparticles also often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example [gold](#) nanoparticles appear deep red to black in solution.

Clay nanoparticles when incorporated into polymer matrices increase reinforcement, leading to stronger plastics, verifiable by a higher [glass transition temperature](#) and other mechanical property tests. These nanoparticles are hard, and impart their properties to the polymer (plastic). Nanoparticles have also been attached to textile fibers in order to create smart and functional clothing.

[Metal](#), [dielectric](#), and [semiconductor](#) nanoparticles have been formed, as well as

[hybrid structures](#) (e.g., core-shell nanoparticles). Nanoparticles made of semiconducting material may also be labeled [quantum dots](#) if they are small enough (typically sub 10 nm) that [quantization](#) of electronic [energy levels](#) occurs. Such nanoscale particles are used in biomedical applications as [drug carriers](#) or [imaging agents](#).

Semi-solid and soft nanoparticles have been manufactured. A prototype nanoparticle of semi-solid nature is the [liposome](#). Various types of liposome nanoparticles are currently used clinically as delivery systems for anticancer drugs and vaccines.

1.3 Synthesis

There are several methods for creating nanoparticles, including both [attrition](#) and [pyrolysis](#). In attrition, macro or micro scale particles are ground in a [ball mill](#), a planetary [ball mill](#), or other size reducing mechanism. The resulting particles are air classified to recover nanoparticles. In pyrolysis, a vaporous precursor (liquid or gas) is forced through an orifice at high pressure and burned. The resulting powder (a version of soot) is air classified to recover oxide particles from by-product gases. Pyrolysis often results in aggregates and agglomerates rather than singleton primary particles.

Thermal plasma can also deliver the energy necessary to cause evaporation of small micrometer size particles. The thermal plasma temperatures are in the order of 10,000 K, so that solid powder easily evaporates. Nanoparticles are formed upon cooling while exiting the plasma region. The main types of the thermal plasma torches used to produce nanoparticles are dc plasma jet, dc arc plasma and radio frequency (RF) induction plasmas. In the arc plasma reactors, the energy necessary for evaporation and reaction is provided by an electric arc which is formed between the anode and the cathode. For example, silica sand can be vaporized with an arc plasma at atmospheric pressure. The resulting mixture of plasma gas and silica vapour can be rapidly cooled by quenching with oxygen, thus ensuring the quality of the fumed silica produced. In RF induction plasma torches, energy coupling to the plasma is

accomplished through the electromagnetic field generated by the induction coil. The plasma gas does not come in contact with electrodes, thus eliminating possible sources of contamination and allowing the operation of such plasma torches with a wide range of gases including inert, reducing, oxidizing and other corrosive atmospheres.

The working frequency is typically between 200 kHz and 40 MHz. Laboratory units run at power levels in the order of 30-50 kW while the large scale industrial units have been tested at power levels up to 1 MW. As the residence time of the injected feed droplets in the plasma is very short it is important that the droplet sizes are small enough in order to obtain complete evaporation. The RF plasma method has been used to synthesize different nanoparticle materials, for example synthesis of various ceramic nanoparticles such as oxides, carbours/carbides and nitrides of Ti and Si.

Inert-gas condensation is frequently used to make nanoparticles from metals with low melting points. The metal is vaporized in a vacuum chamber and then supercooled with an inert gas stream. The supercooled metal vapor condenses in to nanometer-sized particles, which can be entrained in the inert gas stream and deposited on a substrate or studied in situ.

1.4 Characterization

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from [materials science](#). Common techniques are [electron microscopy](#) (SEM), [atomic force microscopy](#) (AFM), [dynamic light scattering](#) (DLS), [x-ray photoelectron spectroscopy](#) (XPS), [powder X-ray diffraction](#) (XRD), [Fourier transform infrared spectroscopy](#) (FTIR), [matrix-assisted laser desorption/ionizationtime-of-flight mass spectrometry](#) (MALDI-TOF), [ultraviolet-visible spectroscopy](#), [dual polarisation interferometry](#) and [nuclear magnetic resonance](#) (NMR).

Therefore, the present investigation was focused on "Synthesis and characterization of titanium nanoparticles from fruits of *zingiber* and *amla* and

studies on their antimicrobial, cytotoxic ”

CHAPTER 2

2.1 LITERATURE SURVEY

Hypersaline conditions are home to species known as , which love salt. Prokaryotic and eukaryotic microorganisms with the ability to manage osmotic pressure and withstand the denaturing effects of high salt concentrations make up the majority of the group. Typically, osmosis causes organisms that live in salty to lose water and perish. need their cytoplasm to be isotonic with their surroundings in order to thrive in salt-rich. They employ two distinct strategies to get there. To endure osmotic stress, the first type of organism (mostly bacteria, some archaea, yeasts, algae, and fungi) stores organic molecules in its cytoplasm .

Neutral amino acids and sugars are the solutes that are most frequently utilised in this process (Stolp, 1998). This approach has a significant drawback in that it makes the organism expend a lot of energy. The second, less frequent adaptation to salt, involves the cytoplasmic uptake of potassium (K^+) ions on a selective basis. In exchange, the organism uses the sodium-potassium pump to expel sodium (Na^+) ions (Cavicchioli ef

al., 2007). However, sodium ions are employed less frequently than potassium ions .

Only one family of Archaea and one order of bacteria exploit this adaption (Kato, 1977). This method has the benefit of using less energy than the adaptation stated above. The fundamental drawback of this strategy is that it requires more work than using suitable solutes because it requires adapting all of the cell's machinery (enzymes, structural proteins, etc.) to high amounts of non-organic ions and salt. Though a few are capable of doing both, the majority of only employ one of these two strategies

CHAPTER 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

2.2 Aim

To synthesize, characterize the titanium nanoparticles using fruits of *zingiber* and *amla* to study their antimicrobial effects.

2.3 Aim and Scope of the Present Investigation

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. titanium nanoparticles exhibit antimicrobial activity. The use of biomedicine benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of titanium nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. By taking all these views, the present study was designed with the following objectives,

- ★ To synthesize titanium nanoparticles using fruits of *zingiber* and *amla*.
- ★ To analyze the synthesized titanium nanoparticles using UV-Visible Spectrophotometer
- ★ To characterize the synthesized titanium nanoparticles using SEM and EDAX.

- ★ To study the antimicrobial effect of the synthesized titanium nanoparticles against *E.coli* and *P.aeruginosa*.
- ★ To study the cytotoxic effects of the synthesized titanium nanoparticles on Vero cells lines.
- ★ To study the antitumor effects of the synthesized titanium nanoparticles on Hep 2 cells lines.

CHAPTER 4

2.4 MATERIALS AND METHODS

The materials used and the methods followed in conduct of various experiments are detailed here.

Preparation of fruit extracts of *zingiber* and *amla*

25g of unripen fruit of *zingiber* and *amla* is weighed and it was crushed without adding water in a pestle and mortar. The content was filtered in a mesh and the filtrate was again passed through what mann no.1 filter paper(pore size 25 μ m). The filtrate then obtained was stored at 4⁰ C for further use.

Preparation of 1mm titanium Nitrate Solution

0.16987g of TiO_2 was weighed and it was dissolved in 1000ml of distilled water in 1000ml conical flask. The preparation was mixed and swirled properly. The flask was then covered with aluminium foil in order to prevent the titanium nitrate solution from photosensitive reaction.

Synthesis of titanium Nanoparticles

1mM zingiber and amla solution of titanium nitrate was prepared and used for the synthesis of titanium nanoparticles. 10 ml of *zingiber and amla* extract was added into 90 ml of zingiber and solution of 1 mM titanium nitrate in 1:9 ratios and kept under sunlight for 4 mins for the reduction into TiO_2 ions.

Analysis of Synthesized titanium Nanoparticles Using UV-Visible Spectrophotometry

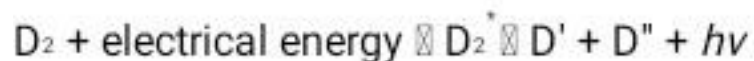
Aim

To measure the absorbance of synthesized titanium nanoparticles under UV-Visible Spectrophotometry.

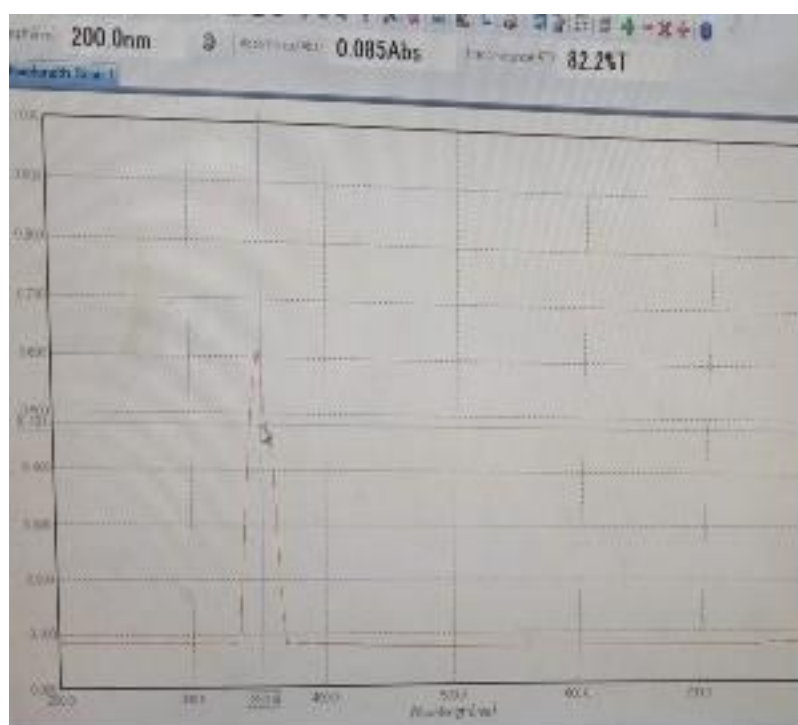
Principle

Sources of UV radiation

It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon. This can be shown as;



Both deuterium and hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.



2.4 Sources of visible radiation

The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the energy output to be stable, the voltage to the lamp must be *very* stable indeed. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability.

Tungsten/halogen lamps contain a small amount of iodine in a quartz "envelope" which also contains the tungsten filament. The iodine reacts with gaseous tungsten, formed by sublimation, producing the volatile compound WI_2 . When molecules of WI_2 hit the filament they decompose, redepositing tungsten back on the filament. The lifetime of a tungsten/halogen lamp is approximately double that of an ordinary tungsten filament lamp. Tungsten/halogen lamps are very efficient, and their output extends well into the ultra-violet. They are used in many modern spectrophotometers.

Wavelength selector (monochromator)

All monochromators contain the following component parts;

- An entrance slit
- A collimating lens

- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

2.5 Cuvettes

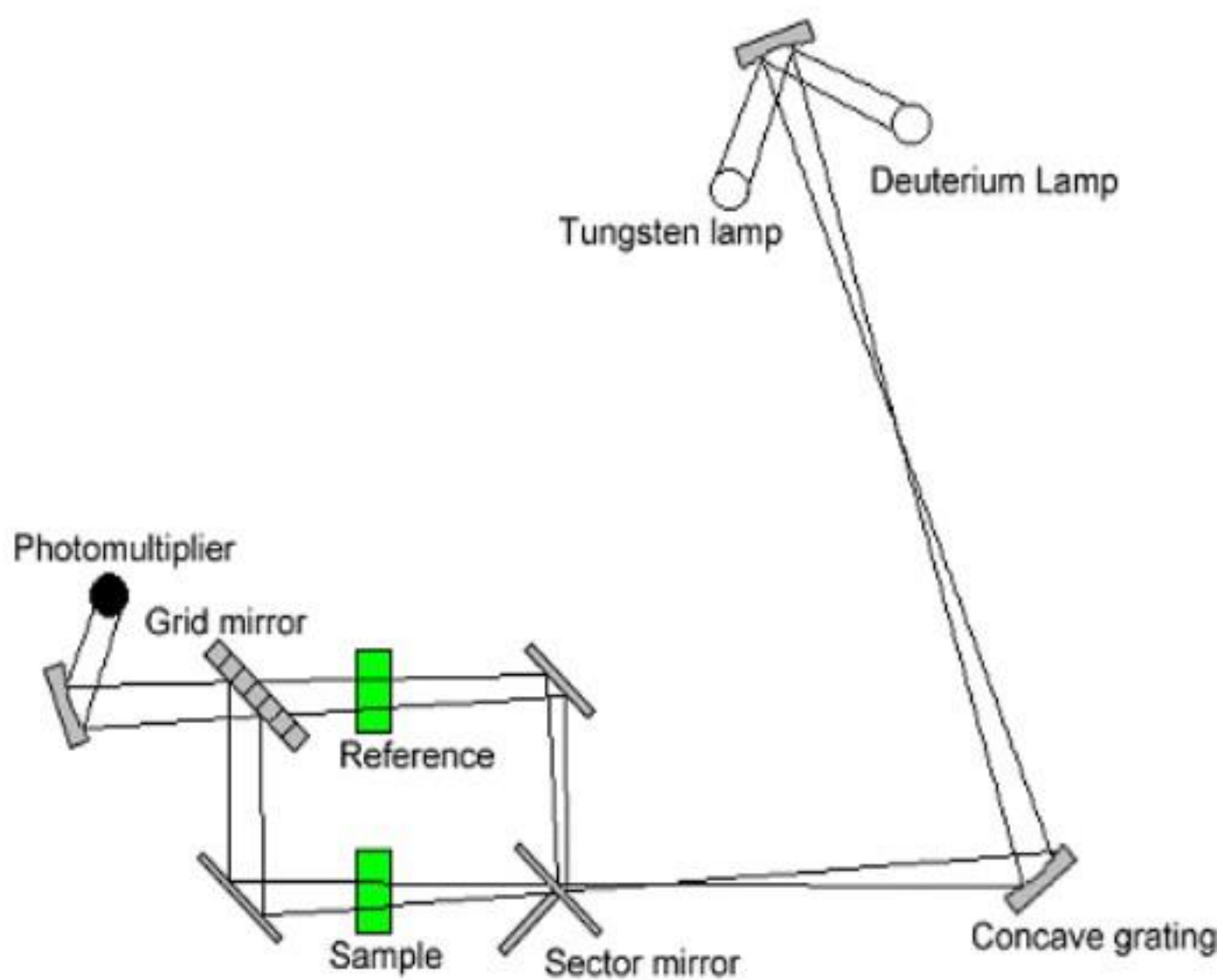
The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

2.6 Detectors

The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It consists of a *photoemissive cathode* (a cathode which emits electrons when struck by photons of radiation), several *dynodes* (which emit several electrons for each electron striking them) and an *anode*.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90V more positive than the cathode). The electrons strike the first dynode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced $10^6 - 10^7$ electrons. The resulting current is amplified and measured.

Figure 2 – Working principle of double beam UV- visible Spectrophotometer



Source:<http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/dbspec2.gif>

4.4.3 Procedure

The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. 1ml of the sample solution was mixed with 2ml of sterile distilled water in a glass quartz cuvette. The contents were pipetted in and out to enhance proper mixing. Fruit extract of *zingiber* and *amlaof* 3ml alone was served as blank. After keeping the blanks in the sample compartment, the baseline correction was made. Out of the two blanks, one blank was replaced with the sample solution and it was scanned from 800nm to 200nm. UV-Vis spectral analysis was done by using double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific).

Characterization of Synthesized titanium Nanoparticles Using SEM and EDAX

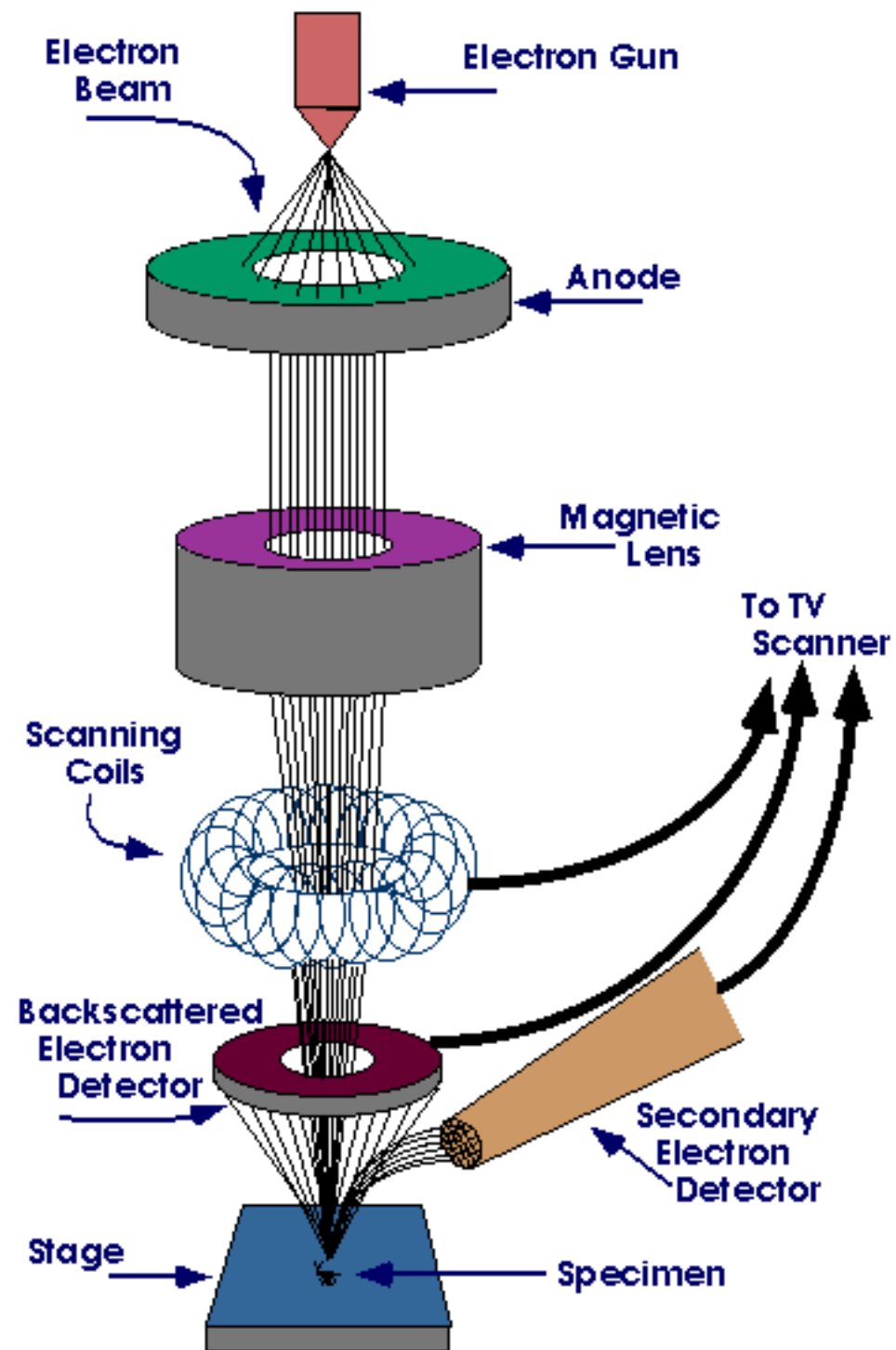
4.4 Aim

To characterize the synthesized titanium nanoparticles using SEM and EDAX

Principle

When an electron from the beam encounters a nucleus in the sample, the resultant Coulombic attraction results in the deflection of the electron's path, known as Rutherford elastic scattering. A few of these electrons will be completely backscattered, re-emerging from the incident surface of the sample. Since the scattering angle is strongly dependent on the atomic number of the nucleus involved, the primary electrons arriving at a given detector position can be used to yield images containing both topological and compositional information. The high energy incident electrons can also interact with the loosely-bound conduction band electrons in the sample. The amount of energy given to these secondary electrons as a result of the interactions is small, and

Figure 3 – Working principle of SEM



Source:<http://www.purdue.edu/rem/rs/graphics/sem2.gif>

So, they have a very limited range in the sample (a few nm). Because of this, only those secondary electrons that are produced within a very short distance of the surface

are able to escape from the sample. This means that this detection mode boasts high resolution topographical images, making this the most widely used of the SEM modes. When the beam is incident on a semiconducting sample, the high energy primary electrons can promote valance band electrons into conduction band via the process of impact ionisation. The resultant electron-hole pairs can then recombine, either by radiative or non-radiative mechanisms. Spectroscopy of the visible and near-visible light emitted from the sample allows information about the radiative recombination processes to be determined, and the use of a scanned beam enables the spatial variations in the luminescence to be mapped. The spatial area of the image collected is dictated by the spacing between adjacent beam points on the specimen. Electronic displacement of the beam allows multiple particle fields to be analyzed without moving the stage; stage motion is reserved for large field displacements. Since, a focused electron beam can be produced only within a relatively high vacuum; the beam, specimen, and detector are housed in a vacuum enclosure. The useful magnification that can be achieved by a SEM is defined by the size of the focused spot projected on the specimen, and this too is ultimately limited by the diffraction of the focused electron beam.

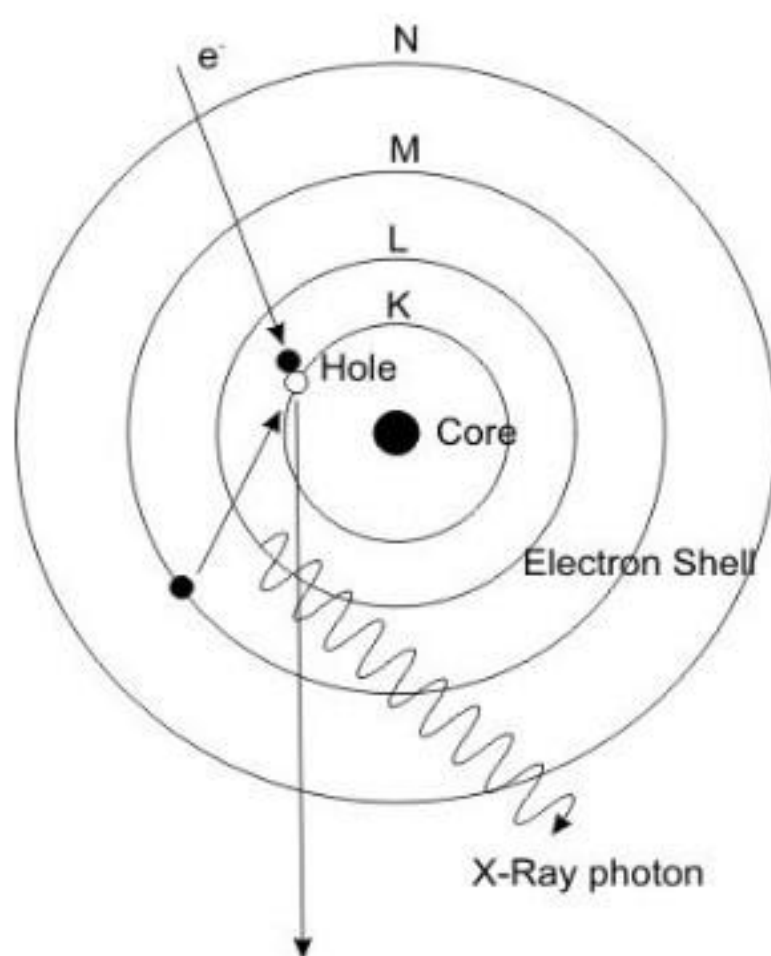
Procedure

Scanning Electron Microscopic (SEM) analysis was done for the synthesized titanium nanoparticle from *zingiber and amlain* in the Department sathyabama institute of science and technology of SEM machine.

EDX

A beam of electrons can interact with an atom is by the ionisation of an inner shell electron. The resultant vacancy is filled by an outer electron, which can release it's energy either via an Augerelectron or (more importantly here) by emitting an X-ray. This produces characteristic lines in the X-ray spectrum corresponding to the electronic transitions involved. Since these lines are specific to a given element, the composition

Figure 4 – Working principle of EDX



Source - <http://www.globalsino.com/micro/1/images/micro9999.jpg>

of the material can be deduced. This can be used to provide quantitative information

about the elements present at a given point on the sample, or alternatively it is possible to map the abundance of a particular element as a function of position.

Procedure

EDX analysis was done for the synthesized titanium nanoparticle from the Department of biotechnology, Chennai.

Antimicrobial Studies

The antibacterial assays were done on *Escherichia coli* and *Pseudomonas aeruginosa* by diffusion method. Briefly, Nutrient broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (100 µl) of each culture were spread on to nutrient agar plates. Six wells, one at the centre and five around the centre well were punched using the cork borer. In the centre well, standard erythromycin antibiotic disc of 15 µg was placed and the surrounding wells were loaded with 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of the synthesized nanoparticle solutions from *zingiber* and *amla*. The plates were then incubated in the incubator at 37°C for 24 hrs and then they were observed visually for the formation of ZOI.

Cytotoxic Effects

Cytotoxicity is the quality of being [toxic](#) to [cells](#). Treating cells with a cytotoxic compound can result in a variety of cell fates. The cells may undergo [necrosis](#), in which they lose membrane integrity and die rapidly as a result of [cell lysis](#). The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death ([apoptosis](#)). Cytotoxicity can also be monitored using the [MTT](#).

Cytotoxicity assay

In order to study the antitumor activity of synthesized titanium nanoparticles, it is important to determine the cytotoxicity concentration of the synthesized titanium nanoparticles. Cytotoxicity test is defined as the upper limit of the extract concentration, which is not toxic to the cell lines. After the addition of the synthesized silver

nanoparticles, cell death and cell viability was estimated. The result is confirmed by additional metabolic intervention experiment such as MTT assay.

Principle

MTT (3-(4,5-[Dimethylthiazol](#)-2-yl)-2,5-di[phenyl](#)tetrazolium bromide, a yellow [tetrazole](#))

Cells in the exponential phase of growth are exposed to a cytotoxic drug. The duration of exposure is usually determined as the time required for maximal damage to occur, but is also influenced by the stability of the drug. After removal of the drug, the cells are allowed to proliferate for two to three populations doubling times (PDTs) in order to distinguish between cells that remain viable and are capable of proliferation and those that remain viable but cannot proliferate. The number of surviving cells is then determined indirectly by MTT dye reduction. The amount of MTT-formazan produced can be determined spectrophotometrically once the MTT-formazan has been dissolved in a suitable solvent.

A main application allows assessing the viability (cell counting) and the proliferation of [cells](#) ([cell culture assays](#)). It can also be used to determine [cytotoxicity](#) of potential [medicinal](#) agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth.

Materials:

1. Cell cultures
 - Hep -2 cell line
 - Vero cell line
2. DMEM (Dulbecco's modified Eagle's medium)
3. MEM (modified Eagle's medium)
4. 10% fetal bovine serum,

Figure 5 - Metabolization of MTT by a mitochondrial reductase to a formazan salt by viable cells



Source:http://www.biocompare.com/images/bc/006/ArticleImages/71785_Ap_No_Cell_3.jpg

5.1% penicillin streptomycin

6. Cell culture flasks

Cell line maintenance and growth conditions

Hep2 cell line and vero cell line was purchased from NCCS pune. The cell lines were maintained at 37⁰C at 5% CO₂ in CO₂ incubator. Cultures were viewed using an inverted microscope to assess the degree of confluency and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with PBS without Ca²⁺/Mg²⁺ using a volume equivalent to half the volume of culture medium. Trypsin/EDTA was added on to the washed cell monolayer using 1ml per 25 cm² of surface area. Flask was rotated to cover monolayer with trypsin. Flask was returned to the CO₂ incubator and left for 2-10 mins. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. 5 ml of complete media was added and cells were triturated to get the cells in the form of suspension .the cell suspension was centrifuged to get the cell pellet. The cell pellet was diluted and seeded into new flasks. The flasks were incubated in CO₂ for 3 day to get monolayer.

MTT assay - Equipment required:

1. Microtiter plate reader with 650- and 570-nm filters
2. Inverted microscope
3. Multi-channel pipette
4. 37°C incubator
5. Laminar flow hood
6. Microtiter plate (flat-bottomed)
7. Sterile tubes (5 ml)
8. Serological pipettes
9. Sterile pipette tips

MTT Solution: 5 mg/ml MTT in PBS. Solution must be filter sterilized after adding MTT.

MTT Solvent: DMSO.

PROCEDURE:

.1Day one

T-25 flask was trypsinized and 5 ml of complete media was added into the trypsinized cells. 15 ml of sterile falcon tube was centrifuged at 500 rpm in the swinging bucket rotor (~400 x g) for 5 min. the media was removed and the cells were resuspended to 1.0 ml with complete media. The Cells were removed aseptically. They were then counted and recorded in per ml. the cells (CV=CV) were diluted to 75,000 cells per ml. complete media was used to dilute the cells. 100µl of cells (7500 total cells) was added into each well and incubated overnight.

2 Day two

The spent media was removed and fresh media and synthesized titanium nanoparticles of varying concentration were added. The final volume was 200µl per well. The MTT plate was incubated in CO₂ incubator for 4 hrs at 37°C. After 4 hrs, the media and the synthesized silver nanoparticles were removed into the new MTT 96 well plate. 20µl of 5 mg/ml MTT and 80µl of media were added into each well. One set of wells was included with MTT but no cells were added in the control under aseptic condition. The plates were then incubated for 3.5 hours at 37°C in culture hood. The media was removed. The cells were not disturbed and it was not rinsed with PBS. 150µl of DMSO (MTT solvent) was then added. The plates were then covered with aluminium foil and the cells were mixed with DMSO. The absorbance was read at 570 nm with a reference filter at 630 nm.

$$(\%) \text{ Percentage of cytotoxicity} = \frac{(\text{control} - \text{sample}) \times 100}{\text{control}}$$

3 Procedure

The vero cells and the Hep 2 cells were grown in six well plates and the media was removed using the micropipette. The cells were washed with phosphate buffer saline. The cells were then fixed with 1ml of cold ethanol and were incubated for about 5 mins. After fixation, the cells were then washed with distilled water. The fixed cells were then viewed under normal light microscope of radical 40x magnification.

CHAPTER 5

RESULTS AND DISCUSSION

Following are the results obtained from the present investigation. Synthesis of titanium Nanoparticles Fruit extract of *zingiber and amla* was mixed with 1mM titanium nitrate solution (1:9 ratios) and they were incubated under sunlight for 4 mins. As the *zingiber and amla* fruit extract was mixed in the aqueous solution of the titanium ion complex, it started to change colourless to dark brown due to reduction of titanium ion (Plate 1), which indicated the formation of silver nanoparticles in the reaction mixture. The change of colour is due to the excitation of surface plasmon vibrations in silver nanoparticles.

Analysis of Synthesized titanium Nanoparticles Using UV-Visible Spectrophotometer

It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. Double beam UV-Vis spectrophotometer (Spectrascan UV-2700, Thermo Scientific) was used to analyze the samples.

Fruit extract of *zingiber and amla* acted as blank. Small aliquot was prepared by mixing the sample solution to sterile distilled water. It was then scanned under UV from 800nm to 200nm. Graph 1 shows the UV-Vis spectra recorded from the reaction mixture after 2 hours. Absorption spectra of silver nanoparticles formed in the reaction media obtained from *zingiber and amla* has absorbance peak at 420 nm (Graph 1). The broadening of the peak indicated that the titanium nanoparticles synthesized from *zingiber and amla* are polydispersed.

Characterization of Synthesized titanium Nanoparticles Using SEM and EDX

The titanium nanostructure by employing fruit extract of *zingiber and amla* was further confirmed by structural view under the scanning electron microscope (Plate 2) and the possible compounds in each of the sample was identified using EDX (Graph 2).

Using EDX, the percentage of titanium present in the synthesized titanium nanoparticle solution from *zingiber and amla* 38.12%.

Antimicrobial Studies

Further, the nanoparticles synthesized by green route are found toxic against *E.coli*, *P. aeruginosa*, *S.aureus* and *S.typhi* at a concentration of 50 µl (Plates 3, 4, 5 and 6). titanium nanoparticles exhibited antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* as it showed a clear inhibition zone and they were compared with the standard antibiotics like erythromycin disc of 15 µg.

Plate

The control, erythromycin disc had formed ZOI of 12 mm diameter against *E.coli*, Whereas the synthesized titanium nanoparticles from *zingiber* for 10 µl, 20 µl, 30 µl, 40 µl and 50 µl was showing ZOI of 11mm, 11mm, 11mm, 11mm and 12.5mm respectively. When compared to the control, nanoparticles are showing better zone of inhibition with 50 µl concentration against *E.coli*.

Plate

The control, erythromycin disc had formed ZOI of 13 mm diameter against *Pseudomonas aeruginosa*, Whereas the synthesized titanium nanoparticles from *Ananas comosus* for 10 µl, 20 µl, 30 µl, 40 µl and 50 µl was showing ZOI of 15mm, 15mm, 15mm, 15mm and 16mm respectively. When compared to the control, nanoparticles are showing better zone of inhibition with 50 µl concentration against *Pseudomonas aeruginosa*.

Plate

The control, erythromycin disc had formed ZOI of 32 mm diameter against *S.aureus*, Whereas the synthesized titanium nanoparticles from *zingiber and amla* for 10 µl, 20 µl, 30 µl, 40 µl and 50 µl was showing ZOI of 14mm, 14.5mm, 15mm, 17mm and 18mm respectively. When compared to the control, nanoparticles are showing less zone of inhibition with 50 µl concentration against *S.aureus*.

Plate

The control, erythromycin disc had formed ZOI of 33 mm diameter against *S.typhi*, Whereas the synthesized titaniumnanoparticles from *zingiber and amla* for 10 µl, 20 µl, 30 µl, 40 µl and 50 µl was showing ZOI of 8mm, 9mm, 9mm, 10mm and 10mm respectively. When compared to the control, nanoparticles are showing less zone of inhibition with 50 µl concentration against *S.typhi*.

Cytotoxic Effects

The MTT assay is one of the [colorimetric assays](#) for measuring the activity of enzymes that reduce MTT or close dyes (XTT, MTS, WSTs) to [formazan](#) dyes, giving a purple color.

The synthesized titanium nanoparticles from fruits of zingiber and amla was treated with both Vero cells and Hep2 cells with varying concentration such as 0.0045µg, 0.009µg, 0.0195µg, 0.039µg, 0.078µg, 0.156µg, 0.3125µg, 0.625µg, 1.25µg, 2.5µg and 5µg (Plates 7 and 8). The untreated vero cells and hep2 cells served as control (Plates 7a and 8a).

Cytotoxic and antitumor effects of titanium nanoparticles synthesized from zingiber and amla

The cytotoxicity of vero cell lines was having a IC_{50} value of 1.25 µg when it was treated with titanium nanoparticles from *amla and zingiber* whereas the cytotoxicity of Hep2 cell was showing a IC_{50} of 0.625 µg. This study says that when the titanium nanoparticles treated can cause cytotoxic effect for Hep2 cells at 0.625 µg, where the same concentration would not affect the normal vero cells (Table 2, Graph 4, Plates 7b and 8b).

Plate 1 – Synthesis of titanium nanoparticles from *zingiber and amla*

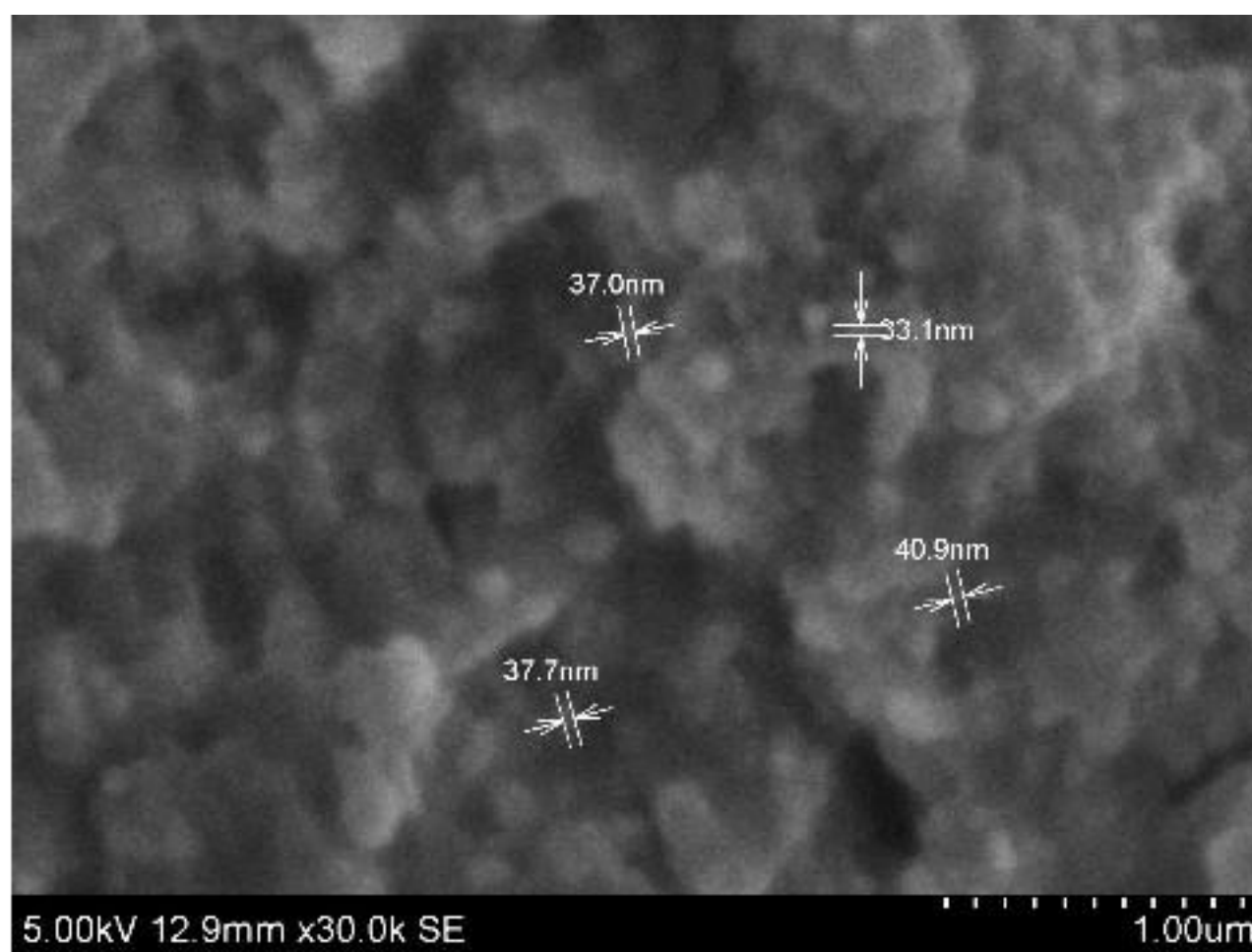
1 2 3

Tube 1-Fruit filtrate of *zingiber and amla*

Tube 2-1mM titanium nitrate solution

Tube 3-1ml *zingiber and amla* filtrate + 9ml tio_2 (after four mins under sunlight)

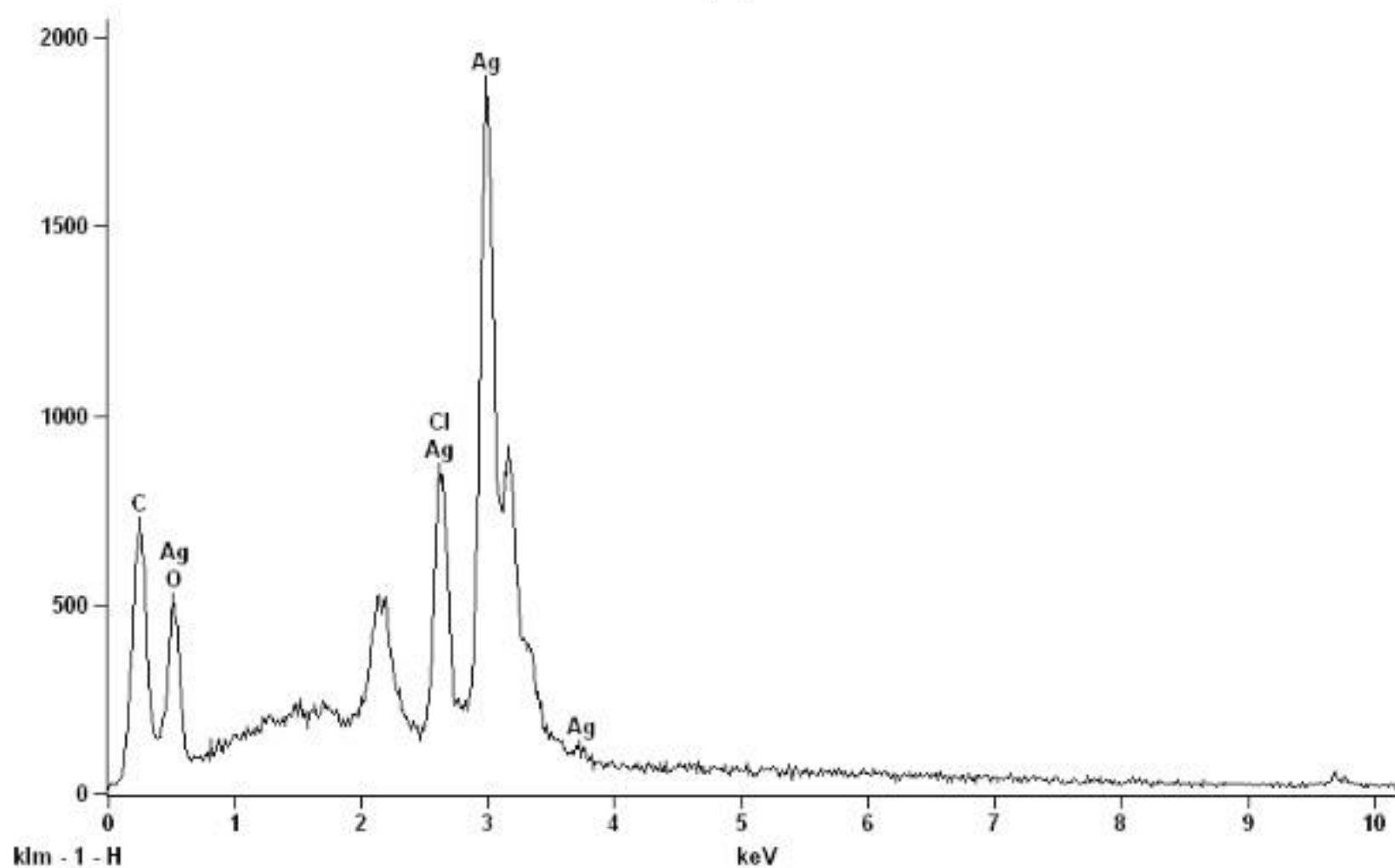
Plate 2 – SEM image of titanium nanoparticles synthesized from *zingiber and amla*



Graph 2 – EDAX analysis of titanium and other compounds from *zingiber and amla*

Full scale counts: 1896

Base(74)



Live Time: 120.0 sec.

Acc.Voltage: 15.0 kV Take Off Angle: 34.6 deg.

Quantitative Results for: Base(74)

Element	Net Counts	Net Counts Error	Weight %	Atom %	Formula	Compnd %
C	5573	+/- 258	33.51	60.09	C	33.51
O	4276	+/- 360	21.19	28.52	O	21.19
Cl	9540	+/- 777	5.75	3.49	Cl	5.75
Cl	1972	+/- 156	—	—		—
Ag	37572	+/- 1950	38.12	7.90	Ag	38.12
Ag	0	+/- 789	—	—		—
Total			100.00	100.00		100.00

Antimicrobial effect of synthesized titanium nanoparticles from *zingiber* and *amla* on *Escherichia coli*



Well c- 15µg of erythromycin antibiotic disc

Well 1- 10µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

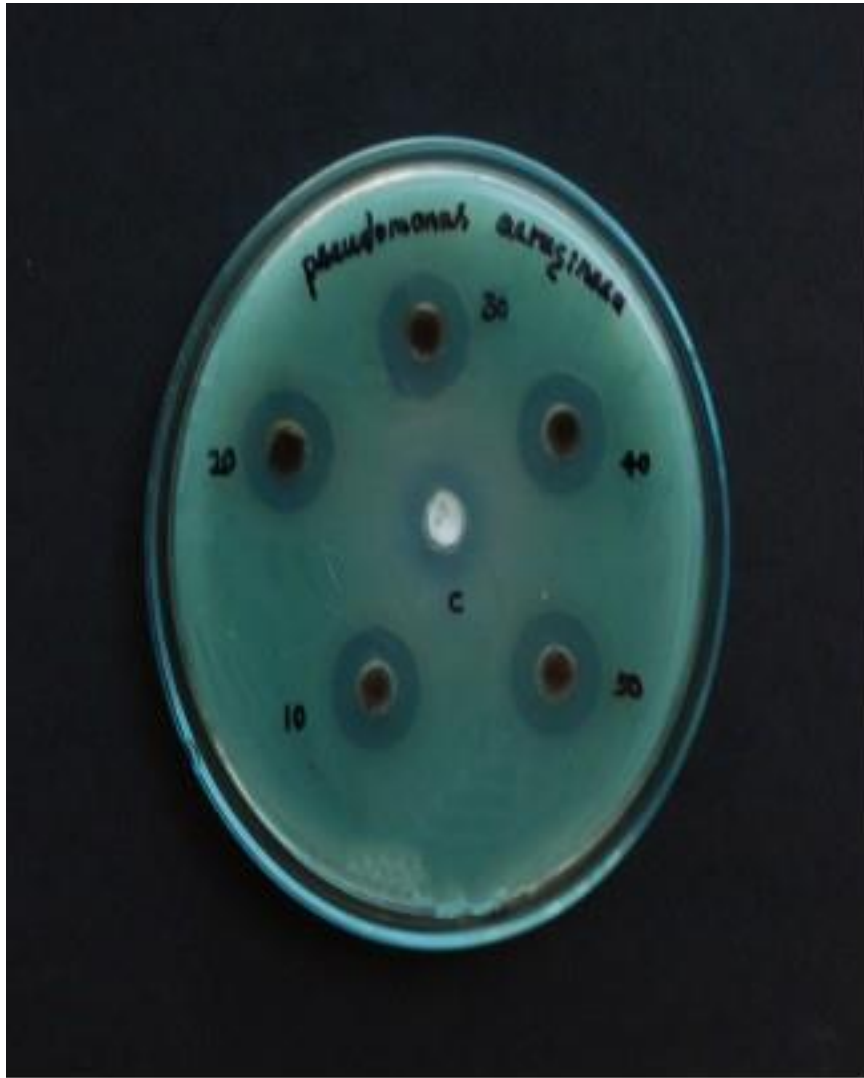
Well 2- 20µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 3- 30µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 4- 40µ of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 5- 50µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Antimicrobial effect of synthesized titanium nanoparticles from *zingiber and amla* on *Pseudomonas aeruginosa*



Well c- 15µg of erythromycin antibiotic disc

Well 1- 10µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

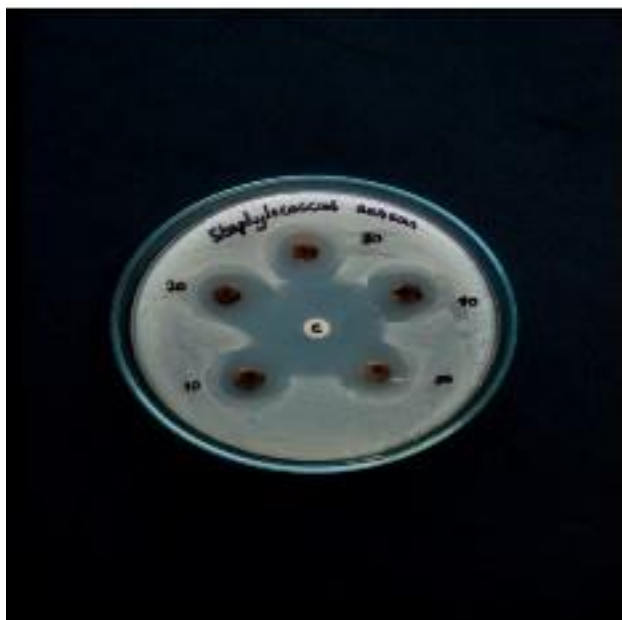
Well 2- 20µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 3- 30µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 4- 40µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 5- 50µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Antimicrobial effects of synthesized titanium nanoparticles from *zingiber and amla* on *Staphylococcus aureus*



Well C- 15µg of erythromycin antibiotic disc

Well 1- 10µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 2- 20µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

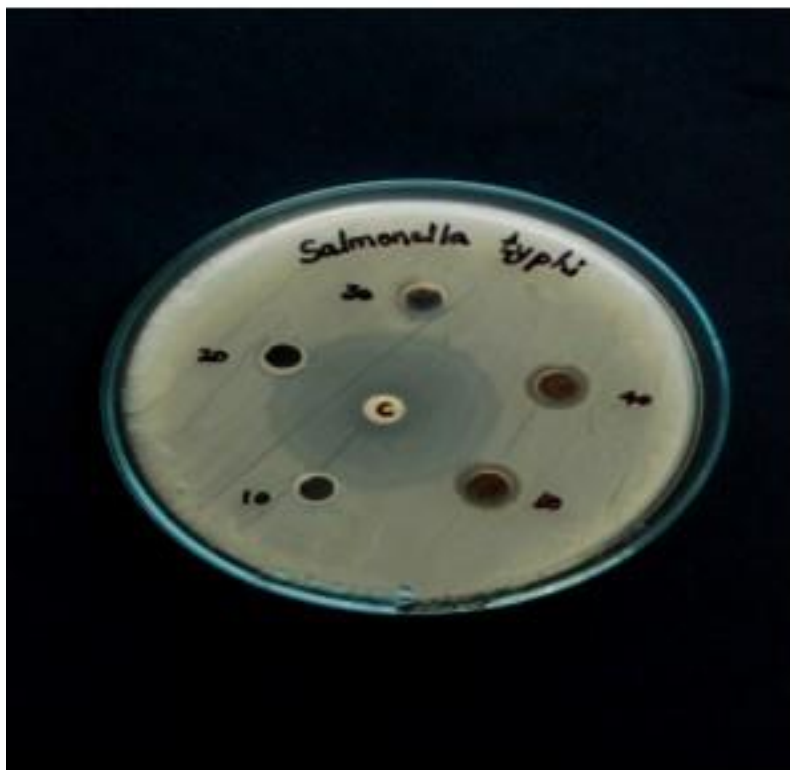
Well 3- 30µl of synthesized titanium nanoparticle solution (from *zingiber and*

amla)

Well 4- 40 μ l of synthesized titanium nanoparticle solution (from *zingiber* and *amla*)

Well 5- 50 μ l of synthesized titanium nanoparticle solution (from *zingiber* and *amla*)

Antimicrobial effects of synthesized titanium nanoparticles from *zingiber* and *amla* on *Salmonella typhi*



Well c- 15 μ g of erythromycin antibiotic disc

Well 1- 10µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 2- 20µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 3- 30µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 4- 40µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Well 5- 50µl of synthesized titanium nanoparticle solution (from *zingiber and amla*)

Table 1 – ZOI (mm) formed by synthesized titanium nanoparticles from *zingiber and amla* against varying strains of microorganisms

	a (mm)	b (mm)	c (mm)	d (mm)
Control (15 µg)	12	13	32	33
10 µl	11	15	14	8

20 μ l	11	15	14.5	9
30 μ l	11	15	15	9
40 μ l	11	15	17	10
50 μ l	12.5	16	18	10

Control -15 μ g Erythromycin disc

a-ZOI (mm) formed by *zingiber and amla* against *E.coli*

b-ZOI (mm) formed by *zingiber and amla* against *P.aeruginosa*

c-ZOI (mm) formed by *zingiber and amla* against *S.aureus*

d-ZOI (mm) formed by *zingiber and amla* against *S.typhi*

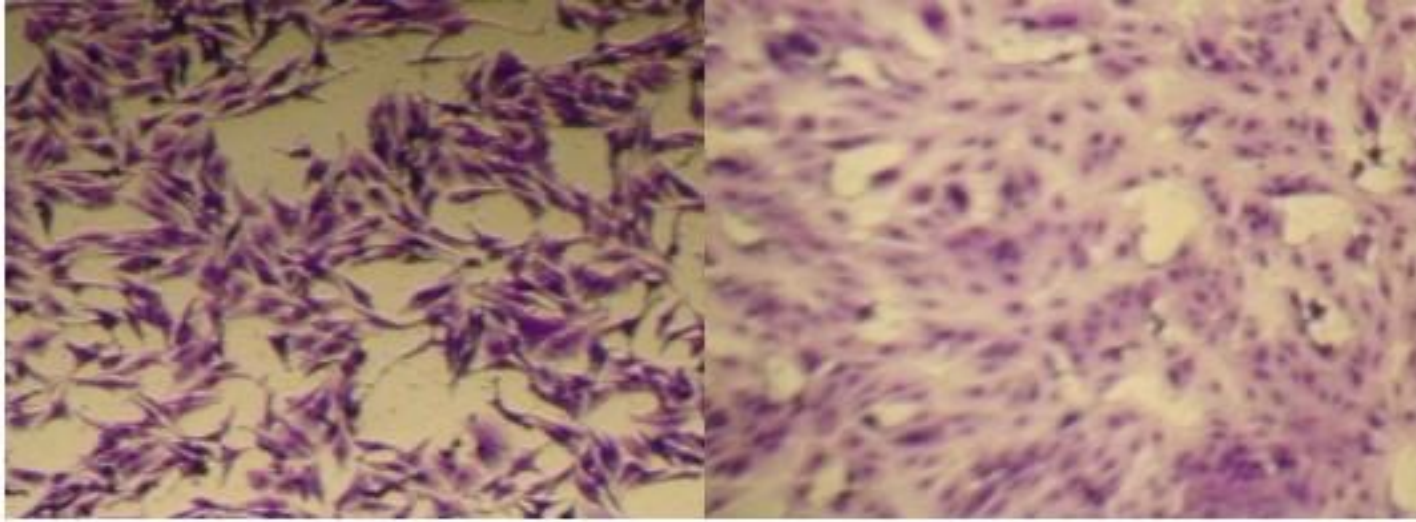
Table 2– Viability of Vero cells and Hep 2 cells when treated with varying concentration of the synthesized titanium nanoparticles from *zingiber and amla* and their

corresponding absorbance in ELISA reader

Concentration(μ g)	Cell viability (vero)	O.D	Cell viability (hep2)	O.D
5	41	0.16	36	0.295
2.5	44	0.171	40	0.328
1.25	50	0.195	48	0.394
0.625	54	0.21	50	0.41
0.3125	58	0.226	54	0.443
0.156	62	0.241	60	0.492
0.078	66	0.257	63	0.512
0.039	70	0.272	67	0.549
0.0195	78	0.303	73	0.599
0.009	84	0.327	80	0.656
0.0045	100	0.257	100	0.82

$IC_{50} = 1.25 \mu g$ $IC_{50} = 0.625 \mu g$

Plate 7 – Untreated Vero cells and treated Vero cells with synthesized titanium nanoparticles from fruits of *zingiber* and *amla* under radical light microscope of 40x magnification

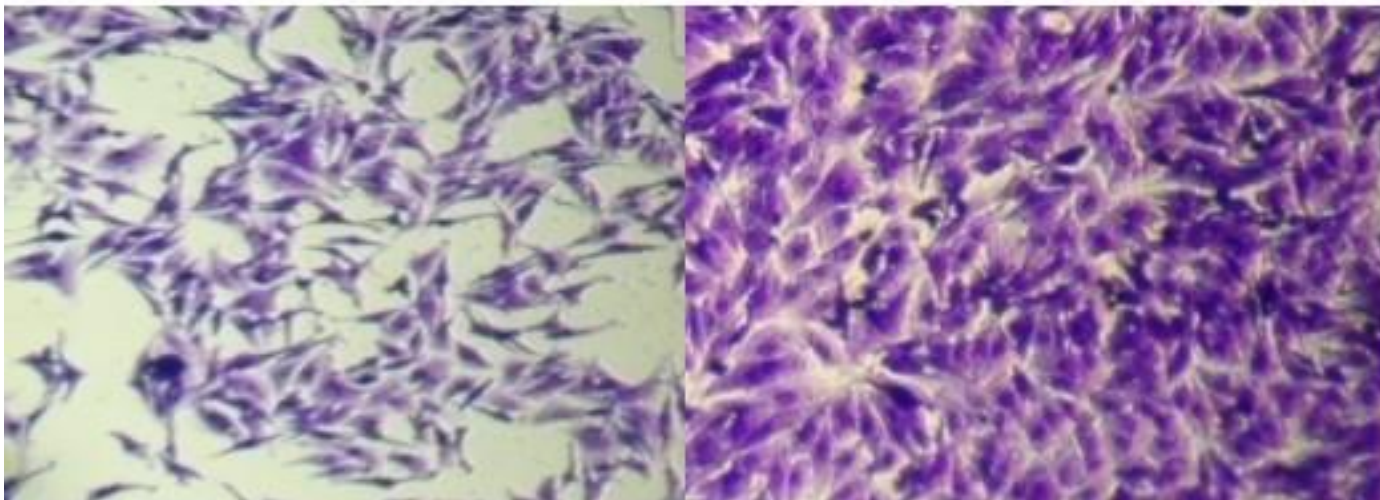


7 a7 b

Plate 7 a – Untreated Vero cells under light microscope of radical 40x magnification

Plate 7 b – Action of synthesized titanium nanoparticles from amla and zingiber Vero cells under light microscope of radical 40x magnification

Plate 8 – Untreated Hep 2 cells and treated Hep 2 cells with synthesized titanium nanoparticles from fruits of zingiber under radical light microscope of 40x magnification



8a 8 b

Plate 8 a – Untreated Hep 2 cells under radical light microscope of 40x magnification

Plate 8 b – Action of synthesized titanium nanoparticles from *amla and zingiber* on Hep 2 cells radical under light microscope of 40x magnification

CHAPTER 6

SUMMARY AND CONCLUSION

Following are the highlights emanated from the investigation.

- ★ The titanium nanoparticles were synthesized from the fruits of *zingiber and amla* and the synthesized silver nanoparticles were detected by observing the change of colour from colourless to dark brown.
- ★ While observing the synthesized titanium nanoparticles solutions under UV-Visible double beam Spectrophotometer, there was broadening of the peak for the sample at 413nm. This says that all the synthesized nanoparticles are not of uniform size. They are polydispersed silver nanoparticles.
- ★ The titanium nanoparticles synthesized from *zingiber and amla* was characterized using SEM in the Department of Biotechnology , and the size of titanium nanoparticles ranged between 37nm and 41nm. Using EDX, the percentage of silver present in the synthesized titanium nanoparticle solution

from *zingiber* and *amla* 38.12%.

- ★ The titanium nanoparticles synthesized from *zingiber* and *amla* when treated against *E.coli*, *P.aeruginosa*, *S.aureus* and *S.typhi* was showing the maximum ZOI as 12.5mm, 16mm, 18mm and 10mm respectively. The concentration present in 50 µl each of the sample loaded is 1.69 µg. Out of this, the titanium nanoparticles synthesized was effective against *S.aureus* and *P.aeruginosa* and less effective against *E.coli* and *S.typhi*.
- ★ The titanium nanoparticles synthesized from *zingiber* and *amla* was showing the cytotoxicity on the vero cell lines and the Hep2 cell lines at an IC_{50} value of 1.25 µg and 0.625 µg respectively. Hence, the study says that the silver nanoparticles synthesized from *zingiber* and *amla* was effective against Hep2 cells at 0.625 µg. at these concentration, they were not lethal to the normal vero cells.

Without adding any chemicals or applying any voltage and pressure, the titanium nanoparticles had been synthesized from pineapple fruit. These biological synthesized nanoparticles were confirmed by measuring the absorbance under UV-Visible Spectrophotometer and the silver nanoparticles were also characterized using SEM and EDAX.

The antimicrobial studies of the synthesized titanium nanoparticles is promising against *S.aureus* and *P.aeruginosa* and also less effect was observed against *E.coli* and *S.typhi*, when compared with the control, erythromycin. The cytotoxic and the antitumor activity were also investigated with the vero and Hep2 cell lines respectively. Therefore, the present investigation concludes that the green synthesized titanium nanoparticles are one of the potent tools as antimicrobial and antitumor agent.

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