

BIO WASTE MEDIATED SYNTHESIS OF SILVER NANO PARTICLES AND ITS EFFECTS ON FOOD PRESERVATION

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

By

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Accredited with Grade "A" by NAAC
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APRIL - 2021



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This is to certify that this Project Report is the bonafide work **G SAI THANUJ (37230072)** who carried out the project entitled “**BIO WASTE MEDIATED SYNTHESIS OF SILVER NANO PARTICLES AND ITS EFFECTS ON FOOD PRESERVATION**” under our supervision from October 2020 to March 2021

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DECLARATION

I, **G SAI THANUJ (37230072)** here by declare that the Project Report entitled **“BIO WASTE MEDIATED SYNTHESIS OF SILVER NANO PARTICLES AND ITS EFFECTS ON FOOD PRESERVATION ”** done by us under the guidance of **Dr .PRABAVATHY** (sathyabama institute of science and technology) **and Dr. ANNAMALAI ,I A S** ,(Director of Avigen Biotech pvt. Ltd)is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Biowaste are environmentally friendly to generate typical nano particles with distinct size, morphology, and chemical composition. nanotechnology is anticipated to transform many areas of food science as there is a rising demand for increased fresh food shelf life as well as the need of protection against food borne diseases urged the development of antimicrobial food packaging.

Stable silver nano particles can be synthesized with maximum absorbance of UV using snow peas peel as biowaste, it is characterized and evaluated by Transmission electron microscopy (TEM), Energy-dispersive X ray (EXD), Fourier-Transform IR spectroscopy (FTIR). Silver nanoparticles (AgNPs), in particular, have an antimicrobial, anti-fungi, anti-yeasts and anti-viral activity and can be combined with both degradable and edible polymers for active food packaging. . Consequently, numerous recent studies have focused on the development of biodegradable packaging materials based on biopolymers. In addition, biopolymers may be classified as active packaging materials, since they have the ability to carry different active substances. Among the most efficient methods, the combination of organic-inorganic, packaging, i.e., polymers (gelatin) embedded silver nanoparticles proved to be highly effective.

thus, persuading for the development of antimicrobial food packing using the biopolymer embedded metal nano particles.

Key words: silver nano particles, shelf life, preservation

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LIST OF ABBREVIATIONS

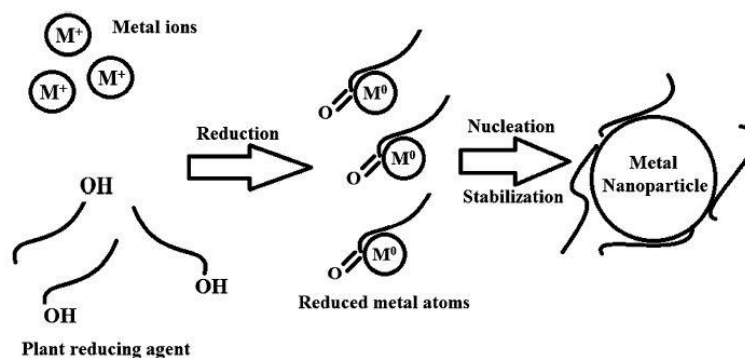
AgNPs	Silver nanoparticles
ST1	CITRUS X SINENIS (orange peel)
ST2	PUNICA GRANATUM (pomegranate peel)
ST3	ARACHIS HYPOGAEA (groundnut shell)
ST4	C. LIMETTA (musambi peel)
ST5	PISUM SATIVUM (pea peel)
UV-VIS	Ultra Violet Visible Spectroscopy
FTIR	Fourier Transform Infra-Red Spectroscopy
HR-TEM	High resolution electron microscope
EDAX	Energy Dispersive X-Ray Analysis
%	Percentage
°C	Degree Celsius
g	Grams
mg	Milligrams
mg/ml	Milligram per milliliter
min	Minute
ml	Milliliter
mm	Millimeter
OD	Optical Density
pH	Potential of hydrogen
ug/ml	Microgram per milliliter

CHAPTER 1

INTRODUCTION

1.1 NANOTECHNOLOGY:

The study of nanotechnology is one of the most energetic lookup areas in modern materials science. Nanoparticles showcase new or multiplied properties based totally on precise traits such as size, distribution, and morphology. The time period nanotechnology used to be coined by way of Professor NORIO TANIGUCHI in 1974. One nanometer is one-billionth or 10^{-9} a meter. Two processes are used to synthesize nanoparticles particularly the bottom-up method and the top-down approach.



Schematic representation of a proposed mechanism of plant-mediated synthesis of metal nanoparticles

Fig (1.1) schematic representation of a proposed mechanism of plant-mediated synthesis of metal nano particle

1.2 METAL NANOPARTICLES:

These are submicron entities that are made out of pure metals specifically gold, silver, platinum, thallium, zinc, iron, titanium, cerium, or their compounds such as oxides, halides, sulphides, phosphates, fluorides, and chlorides. Gold, silver, copper nanoparticles exhibit resonance which indicates clear absorption in the seen region. Ankamwar B, Damle C, Ahmad A, Sastry M, (2005)

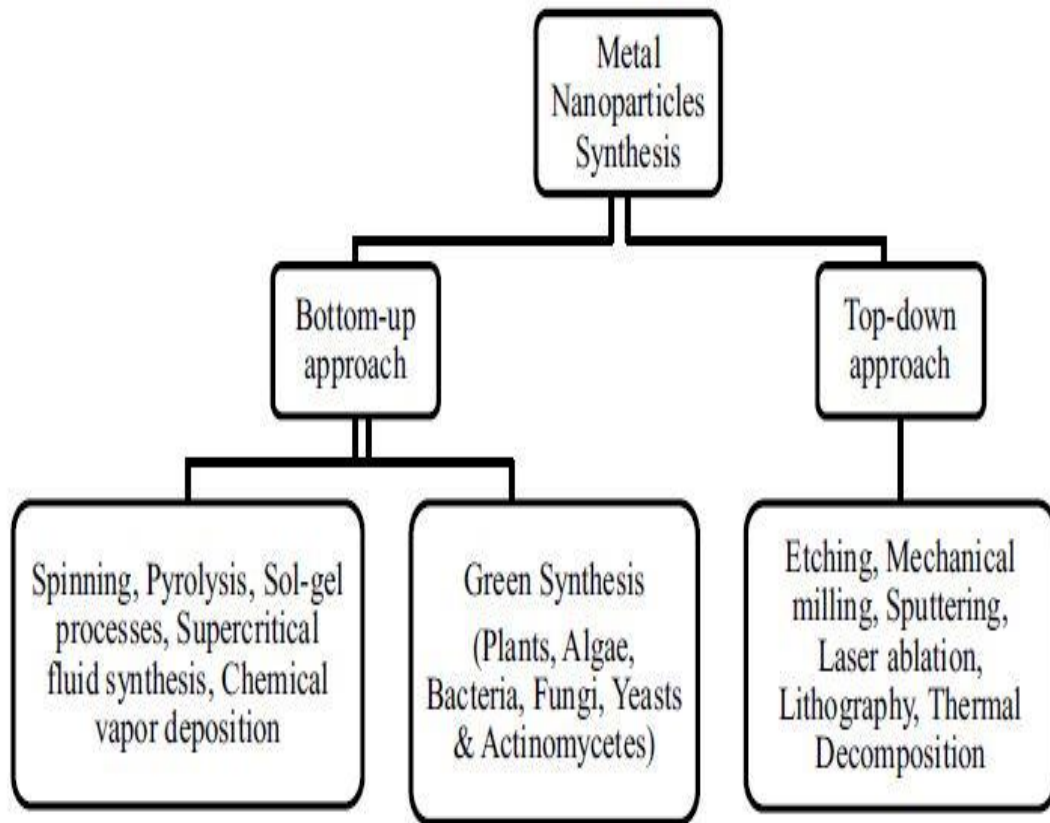


Fig (1.2) approaches of metal nano particles synthesis

1.2.1 Bottom-Up Approach:

The bottom-up method is a nano-architectural phenomenon of self-meeting of substances from cluster-to-cluster, molecule-to-molecule or atom-to-atom on top of a base substrate. The most important issue in the bottom-up strategy is the adhesion of the floor layers to the base substrate. The most generally used bottom-up techniques are welding & riveting. (Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A, (2009).

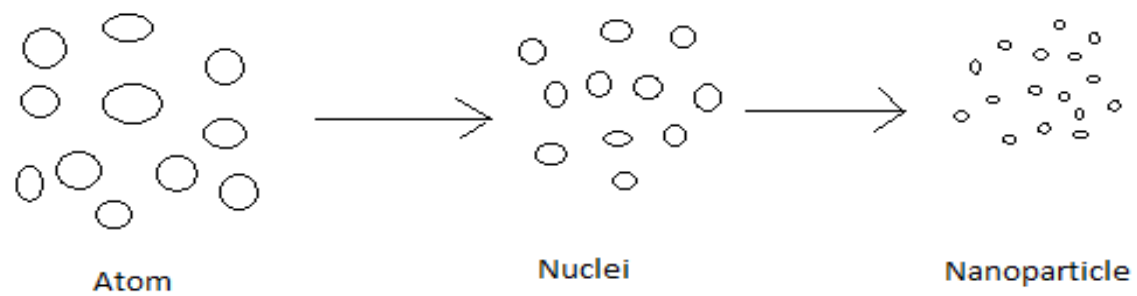


Fig (1.3): Bottom High approach

1.2.2 Top-Down Approach:

The Top-down approach refers to a set of fabrication applied sciences beginning with a block bulk fabric that shares the identical cloth with the base substrate. The most often used top-down strategies are milling, drilling, and grinding.

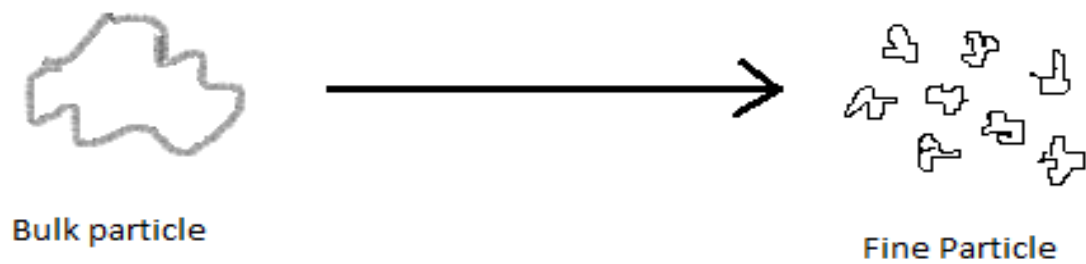


Fig (1.4): Top-down approach

Flow chart of the synthesis of nanoparticles

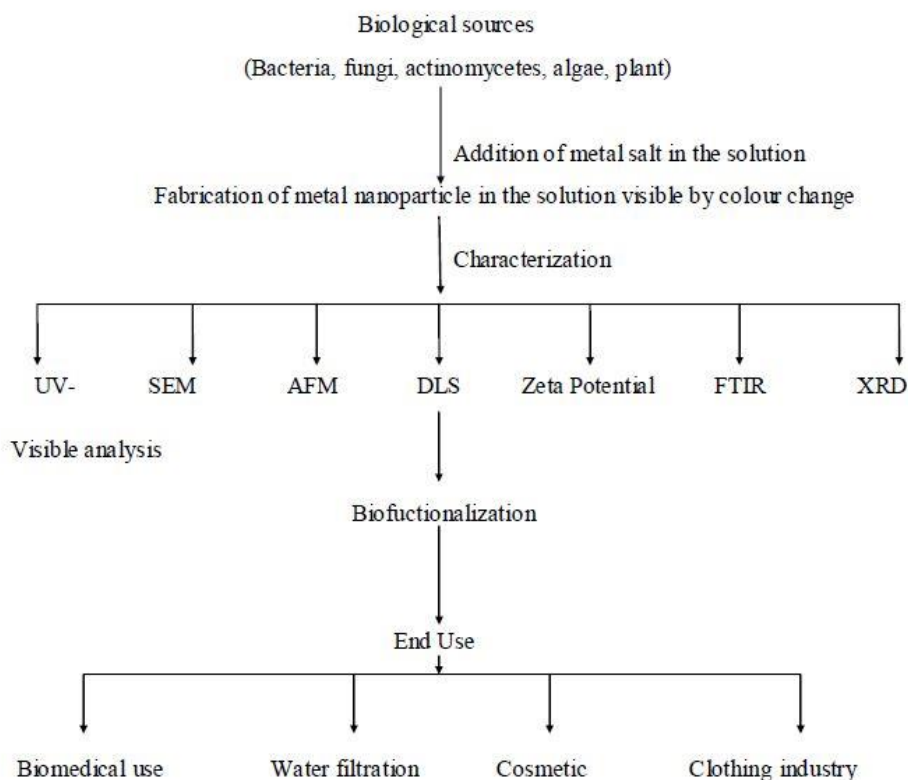


Fig (1.5) Synthesis of Nano Particles with Biological Sources

1.3 SILVERNANOPARTICLES:

Silver is a gleaming, very ductile, and malleable thing however barely tougher than gold, with an image of Ag and an atomic quantity of forty-seven. It is one of the primary factors that make up our planet. In nature, it exists as a native element, as an alloy combining with different metals (e.g., gold), and as minerals (e.g., chlorargyrite and argentite). Chemically, silver possesses 4 specific oxidation states, i.e., Ag⁰, Ag¹⁺, Ag²⁺, and Ag³⁺.

Silver is extensively used as a catalyst for the oxidation of methanol to formaldehyde and ethylene oxide. Due to its colloidal nature, it makes use of as a substrate for surface-enhanced spectroscopy, as it partly requires an electrically conducting surface. In this era, silver is used as an antimicrobial agent. Recent focuses toward

silver nanoparticle synthesis for growing the remedy of antibiotic resistance, prompted with the aid of the misuse of antibiotic. The first commentary of nanoparticle synthesis is the shade exchange of the aqueous solution, if the wavelength of the answer is from 380-400nm then a smaller nanoparticle is formed. This band formation after nanoparticle synthesis is due to collective 15 oscillation or excitation of an electron in the answer (i.e., floor plasmon resonance)

Nanoparticles produced	Biological entity	Size (nm)	References
AgNP	<i>Cycas circinalis</i> , <i>Ficus amplissima</i> , <i>Commelina benghalensis</i> , and <i>Lippia nodiflora</i>	13–51	Johnson and Prabu (2015)
AgNP	<i>Sinapis arvensis</i> seed exudates	1–35	Khatami et al. (2015)
AgNP	<i>Butea monosperma</i> leaf extract	5–30	Chaturvedi and Verma (2015)
AgNP	<i>Musa balbisiana</i> , <i>Azadirachta indica</i> and <i>Ocimum tenuiflorum</i>	20–50	Banerjee et al. (2014)
AgNP	<i>Quercus infectoria</i> extract	40	Heydari and Rashidipour (2015)
AgNP	<i>Schizophyllum commune</i> (mushroom fungus)	51–93	Arun et al. (2014)
AgNP	<i>Lantana camara</i> berry	75.2	Kumar et al. (2015)
AgNP	Pomegranate peel extract	5–50	Shanmugavadivu et al. (2014)
AgNP	<i>Aloe vera</i> extract	500	(SaraHbrahim et al. (2014)
AgNP	<i>Aegle marmelos</i> (Bael) fruit extract	34.7	Nithya Deva Krupa and Raghavan (2014)
AgNP	<i>Bacillus stearothermophilus</i>	14 ± 4	El-Batal et al. (2013)
AgNP	<i>Sargassum muticum</i> (brown marine macroalgae)	5–15	Azizi et al. (2013)
AgNP	<i>Streptomyces</i> sp. LK3	5	Karthik et al. (2014)
AgNP	<i>Glycine max</i> (soybean)	7–29	Sasikala et al. (2012)
AgNP	<i>Schizophyllum radiatum</i> HE863742.1	10–40	Metuku et al. (2014)
AgNP	<i>Tinospora cordifolia</i> leaf extract	3.3–20.6	Rajathi et al. (2012)
AgNP	<i>Fusarium oxysporum</i>	25–50	Birla et al. (2013)
AgNP	<i>Trichoderma reesei</i> (fungus)	5–50	Khabat Vahabi et al. (2011)
AgNP	<i>Sargassum longifolium</i>	30	Saraniya Devi et al. (2013)
AgNP	<i>Gliricidia Sepium</i>	10–50	Raut Rajesh et al. (2009)

Fig 1.6 Uses of various biological entities in the synthesis of silver nanoparticles during the period of 2009–2015

1.4 CHARACTERIZATION OF SILVER NANOPARTICLES:

The characterization finds out about of silver nanoparticle used to be executed via the inspecting size, form and extent of particles. Number of method is used for this purpose, along with UV-visible spectroscopy, Transmission Electron Microscopy (SEM), Fourier Transmission Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD)

1.4.1 UV-VIS SPECTROSCOPY:

Absorbance spectroscopy is used to decide the optical homes of a solution. A Light is despatched thru the pattern answer and the quantity of absorbed mild is measured. When the wavelength is diverse and the absorbance is measured at every wavelength. The absorbance can be used to measure the attention of a answer via the use of Beer-Lamberts Law. The examination of nanoparticles, the optical homes are a lot extra complicated. For instance, the measured absorbance spectrum does no longer always exhibit the genuine absorbance however the extinction of the mild is each the absorbed and the scattered mild from the particles. These wave lengths occur due to the floor Plasmon resonance of the particle.

1.4.2 TRANSMISSION ELECTRON MICROSCOPY:

In NPs characterization, TEM has been extensively used for imaging functions (aimed for visualization of the synthesized NPs and NPs interplay with organism), measurement and structure characterization, etc. Another essential utility of TEM is single particle elemental evaluation of an object on every occasion the TEM is complimented through spectroscopy detector/s

In general, the TEM consists of electron gun, collection of focusing and magnifying lenses, pattern stage and fluorescence display screen or screen for visualization. A warm filament of Tungsten (T 2500-3000 K) and Lanthanum hexaborides (LaB6, T 1400-2000 K) are some examples of electron gun, with accelerated voltage 30 kV-1,000kV or more. An extremely excessive vacuum wishes to be utilized to the indoors of TEM to keep away from the collision of electron with molecules inside the air. The condensing coil(s) focal point the electron beam on a small region of specimen (2-3 μm) positioned in the specimen stage.

Sufficient contrast, a relative difference in intensity between an object and background is necessary to generate an image. Contrast of an image is determined by the nature of the samples and the interaction between the sample and the electron. The high density and thin sample (<100nm) will scatter the electron and creates low intensity, but the lesser density (i.e., background) area of the sample will creates a higher intensity so the contrast is produced. However, for thicker

sample, contribution of electron absorption and multiple scattering will interfere the contrast generation. Thus preparing a very thin sample is very important in EM.

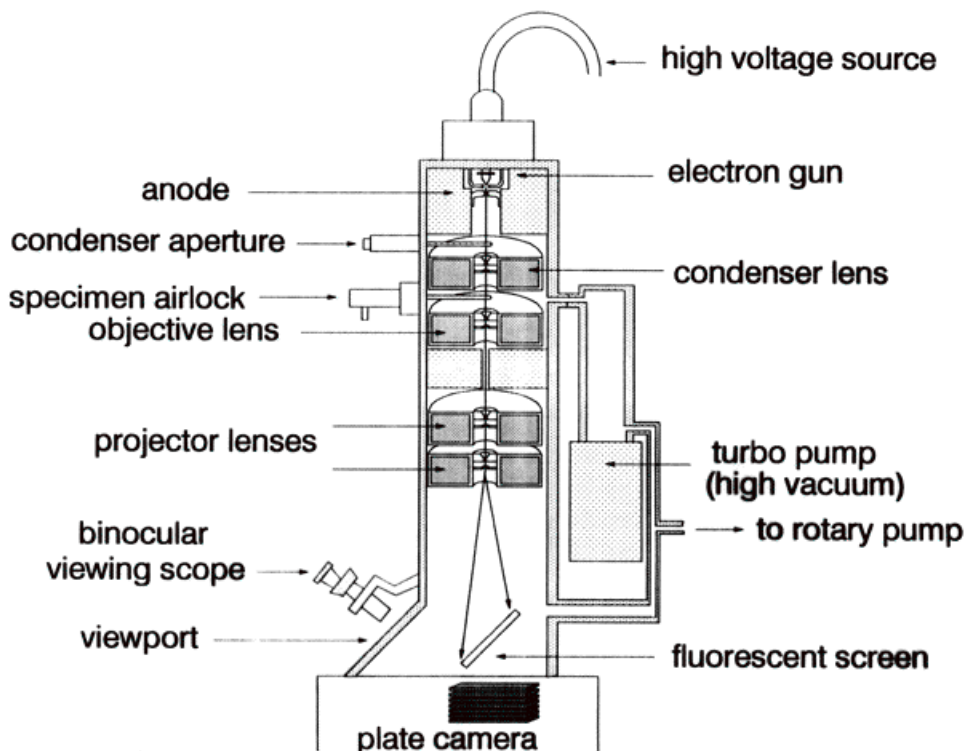


Fig (1.7) Transmission Electron Microscope

1.4.3 FOURIER TRANSMISSION INFRARED SPECTROSCOPY:

FTIR is a chemical analytical approach that measures infrared depth v/s wavelength or wavenumber of lights. It is used to evaluation of viable biomolecules and additionally bonding interactions between themselves. IR spectroscopy detects the vibration traits of chemical purposeful businesses of the sample. When infrared mild interacts with matter, chemical bonds will exhibit stretch, contract, and bend form. This chemical practical crew tends to soak up infrared radiation in a particular wavenumber vary of the shape of the relaxation of the molecule. In the silver nanoparticle synthesis, FTIR records measures interplay between Ag salts and proteins molecules, which correct for the discount of silver ions and stabilization of Ag NPS formed.

1.4.4 X-RAY DIFFRACTION:

XRD is a method to used go find out about the segment composition of a sample, crystal structure, texture, or orientation. The precept of XRD is that the X-rays are surpassed via a cloth and the sample produced offers statistics on the dimension and structure of the unit cell. The atoms are crystal in shape organized in a periodic array and as a result can diffracted mild at a one-of-a-kind angle. When an X-ray passing via a crystal it produces a diffraction pattern, that diffraction offers records about the atomic association inside the crystals. In silver nanoparticles, XRD offers segment shape and purity of the particle.

1.5 IMPORTANCE OF SILVER NANOPARTICLES:

It is used for purification and first-rate administration of air, biosensing, imaging, drug transport system. Biologically synthesized silver nanoparticles have many functions like coatings for photo voltaic power absorption and intercalation cloth for electrical batteries, as optical receptors, as catalysts in chemical reactions, for bio labeling, and as antimicrobials. Though silver nanoparticles are cytotoxic they have great purposes in the discipline of excessive sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis, and micro-electronics. It has some achievable functions like diagnostic biomedical optical imaging, organic implants (like coronary heart valves), and clinical utility like wound dressings, contraceptive devices, surgical instruments, and bone prostheses. Many principal purchaser items producers already This merchandise encompass nano silver-lined refrigerators, air conditioners, and washing machines.

1.6 EFFECTS OF SILVER NANOPARTICLES ON HUMANS:

Metallic silver nanoparticle seems to pose minimal chance to health, whereas soluble silver compounds are greater comfortably absorbed and have the viable to produce unfavourable effects. The huge range of makes use of of silver approves publicity thru quite a number routes of entry into the body. Ingestion is the

fundamental route for entry for silver compounds and colloidal silver proteins. Dietary consumption of silver is estimated at 70- 90µg/day. Since silver in any shape is no longer concept to be poisonous to the immune, cardiovascular, nervous, or reproductive machine and is no longer viewed to be carcinogenic, consequently silver is highly non-toxic. (Cassandra D, N.N., Jodi H, Linfeng G, Tan, Li, et al., "Green synthesis of gold and silver nanoparticles from plant extracts".)

1.7 ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES:

Ag NP is particularly antimicrobial to various species of bacteria, together with the frequent kitchen microbe, E. coli. According to the mechanism reported, silver nanoparticles engage with the outer membrane of microorganism and arrest the breathing and some different metabolic pathway that leads to the loss of life of the bacteria. Silver nanoparticles have been currently recognized to be a promising antimicrobial agent that acts on a wide vary of goal web sites each extracellularly as properly as intracellularly. Silver nanoparticles exhibit very sturdy bactericidal recreation in opposition to gram-positive as nicely as gram-negative microorganism which include multi-resistant strains

- ultra-small sized silver nanoparticles onto the cell wall of bacteria, ensuing in modifications in the cell wall which in flip is unable to guard the indoors of the cell;
- Bacterial boom and proliferation are adversely inhibited by using the adhesion of Through the penetration of silver nanoparticles into the bacterial cell, it leads to DNA damage, or even cell death, with the aid of altering its regular functioning of bacterial DNA; and
- The interplay of Ag⁺ ions with the proteins containing sulphur existing in the bacterial cell wall irreversibly disrupted the bacterial mobile wall. This proposed mechanism is additionally deduced as the essential antibacterial mechanism in evaluating antimicrobial activity.
- Hence there is massive scientific development in the learn about of the organic software of AgNPs

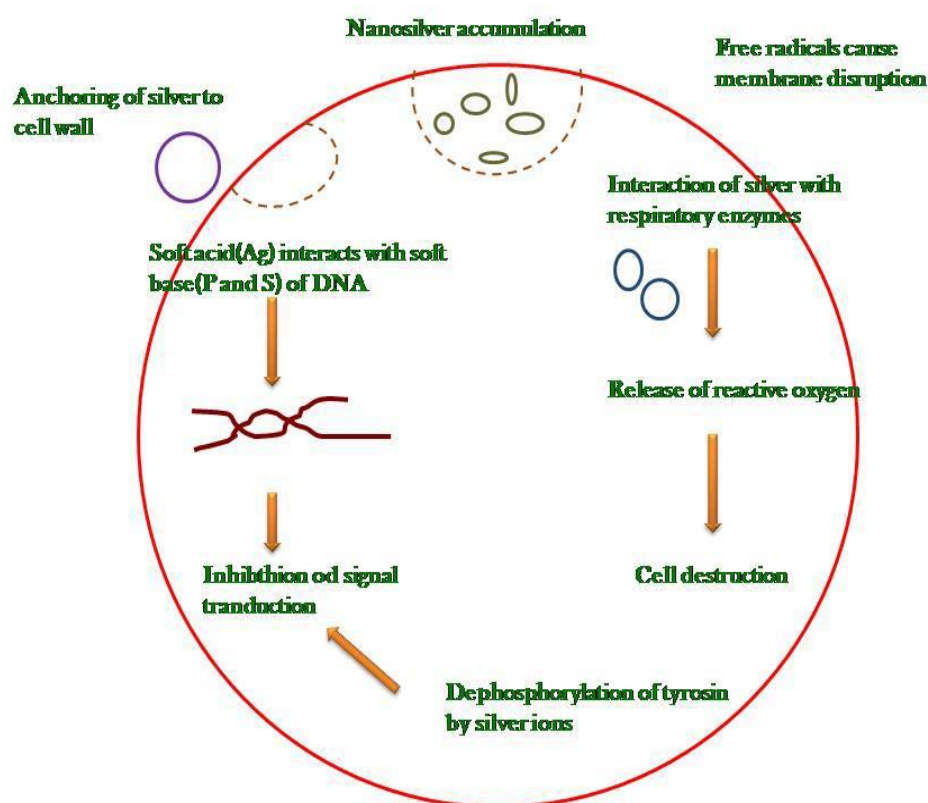


Fig (1.8) anti-bacterial activity

1.8 GELATIN FILM:

Film-forming residences have been substantially used to shield meals throughout its shelf life, as an outer film, from dryness, exhibition to mild and/or exposure to oxygen. Gelatin is an animal protein bought with the aid of a managed hydrolysis of the fibrous insoluble collagen from bones and skins of animals and fish. It is nicely recognized for its film-forming properties. In addition, it is abundantly available, with low cost, and is effortlessly biodegradable and biocompatible. Gelatin films have incredible barrier properties against gas, oxygen and aromas at low or intermediate relative humidity. Gelatin has regularly been used as a appropriate raw fabric to make suitable for eating or biodegradable movies for advantageous meals packaging. Several researchers have used this fabric to make composite movies by way of mixing with different polymers or nano clays, such as chitosan, whey protein, pectin, and shellac

1.9 GELATIN-BASED FILMS AND COATINGS FOR FOOD PACKAGING:

Gelatin-based suitable for eating films and coatings have already been proposed to protect, hold or lengthen the shelf-life of meals products. Factors that must be regarded when designing this kind of device include the chemical nature of food, managed launch mechanisms, meals organoleptic traits and additive toxicity, storage and distribution, bodily and mechanical residences of packaging substances and guidelines to be utilized in this framework. Consequently, exceptional sorts of components should be delivered to enhance or alter the remaining properties in order to reap suitable gelatin-based films or coatings for meals packaging. Recent research have centered on fascinating methods to improve lively packaging videos and coatings, which includes antimicrobial, antioxidant and different marketers which can beautify the organic facets of food. These factors are generally quintessential oils or extracts bought from flora and spices which show off antimicrobial and antioxidant properties, and most of them are regarded to be Generally Recognized as Safe (GRAS). In order to minimize the use of artificial chemical components in the meals industry, the use of herbal meals components with antimicrobial and/or antioxidant residences except bad results on human fitness has accelerated in the ultimate years. These herbal components are in a position to forestall or decrease the deterioration of food brought on via oxidation or microbiological effects, accordingly assisting to keep and lengthen meals shelf-life.

Gelatin	Active additive	application	Main benefits	reference
Gelatin	Silver nanoparticle	film	Enhancement in hydrophobicity, water vapour and UV barrier Compact surface structure Strong antibacterial activity	Kanmani, P.; Rhim, J.W. Physicochemical properties of gelatin/silver nanoparticle antimicrobial composite films. Food Chem. 2014 ,

Table (1.1)

compound used as active additives in gelatin-based films and coatings

CHAPTER 2

LITERATURE SURVEY

MOHAMMED FAYAZ A et al., (2011), In this study, the synthesis and characterization of exopolysaccharide-stabilized silver nanoparticles (AgNPs) was carried out for the degradation of industrial textile dyes. Characterization of AgNPs was done using surface plasmon spectra using UV–Vis spectroscopy, X-ray diffraction (XRD) and Raman spectroscopy. The morphological nature of AgNPs was determined through transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM), which indicated that the AgNPs were spherical in shape, with an average size of 35 nm. The thermal behaviour of AgNPs revealed that it is stable up to 437.1 °C and the required energy is 808.2J/g in TGA-DTA analysis. Ability of EPS stabilized AgNPs for degradation of azo dyes such as Methyl orange (MO) and Congo red (CR) showed that EPS stabilized AgNPs were found to be efficient in facilitating the degradation process of food . The electron transfer takes place from reducing agent to dye molecule via nanoparticles, resulting in the destruction of the dye chromophore structure. This makes EPS-AgNPs a suitable, cheap and environment friendly candidate for biodegradation.

MUBARAKALI D et al., (2011), work on plant extract mediated synthesis of silver and nanoparticles and its antibacterial activity against clinically isolated pathogens. MOHAMMED FAYAZ A et al., (2011), studies on biosynthesis of anisotropic gold nano particles using maducalongi tolia extract and their potential infrared absorption. KUMAR KP et al., (2011), studies on green synthesis of silver nano particles with zingibee officinale extract characterization and blood compatibility. YANG N et al., (2014), work on biosynthesis of gold nano particles using agricultural waste mango peel extract and its invitro cytotoxic effect on two normal cells. PATRA S et al., (2014) studies on green synthesis, characterization silver nanoparticle and their potential application for cancer therapeutics.

BASEVEGOWDA N et al., (2014), GARDEATORRESDEY J et al.,(2002), The bio-molecules from various plant components and microbial species have been used as potential agents for the synthesis of silver nanoparticles (AgNPs). In spite of a wide range of bio-molecules assisting in the process, synthesizing stable and widely applicable AgNPs by many researchers still poses a considerable challenge to the researchers. The biological agents for synthesizing AgNPs cover compounds produced naturally in microbes and plants. More than 100 different biological sources for synthesizing AgNPs are reported in the past decade by various authors. Reaction parameters under which the AgNPs were being synthesized hold prominent impact on their size, shape and application. Available published information on AgNPs synthesis, effects of various parameters, characterization techniques, properties and their application are summarised and critically discussed in this review.

Garg, D., Sarkar, A., Chand, P. et al. Synthesis of silver nanoparticles utilizing various biological systems: mechanisms and applications. The evolving technology of nanoparticle synthesis, especially silver nanoparticle (AgNPs) has already been applied in various fields i.e., electronics, optics, catalysis, food, health and environment. With advancement in research, it is possible to develop nanoparticles of various size, shape, morphology, and surface to volume ratio utilizing biological systems. A number of different agents and methods can be employed to develop choice based AgNPs using algae, plants, fungi and bacteria. The use of plant extracts to produce AgNPs appears to be more convenient, as the method is simple, environmentally friendly and inexpensive, also requiring a single-step. The microbial synthesis of AgNps showed intracellular and extracellular mechanisms to reduce metal ions into nanoparticles. Studies have shown that different size (1–100 nm) and shapes (spherical, triangular and hexagonal etc.) of SHANKAR SS et al., (2003), can be produced from various biological routes and these diverse nanoparticles have various functions and usability i.e., agriculture, medical-science, textile, cosmetics and environment protection. The present review provides an overview of various biological systems used for AgNP synthesis, its underlying mechanisms, further highlighting the current research and applications of variable shape and sized AgNPs.

RODRIGUEZ-LEON et al., (2019), Contamination by fungi and bacteria during the in vitro propagation of plants leads to considerable losses of biological material and precludes phytosanitary certification. The anti-microbial effect of silver nanoparticles (AgNPs) may be an alternative for the eradication of in vitro contaminants. This study evaluated the microbicidal activity of AgNPs on a recurrent fungus during the micropropagation of stevia (*Stevia rebaudiana* Bertoni). First, the fungus was isolated and identified at a molecular level by the sequencing and analysis of the ITS4/ITS5 rDNA region. The results of the phylogenetic analysis of various fungal species showed that the strain under study (16-166-H) belongs to the genus *Sordaria* and is 99.64% similar to *S. tomento-alba* (strain CBS 260.78). Subsequently, the inhibition of the growth of *S. tomento-alba* was tested under different concentrations of AgNPs (0, 25, 50, 100, and 200 mg L⁻¹), observing that 50 and 100 mg L⁻¹ achieve ca. 50% growth inhibition (IC₅₀), while 200 mg L⁻¹ produces a drastic inhibition. On the other hand, the shape and size of AgNPs was examined using transmission electron microscopy (TEM), and the transport and accumulation of AgNPs in *S. tomento-alba* cells were monitored through multiphoton microscopy. The morphological and fluorescence analyses showed that AgNPs display different sizes, with larger nanoparticles retained in fungal cell walls while smaller AgNPs penetrate into fungal cells. Probably, apoplastic and symplastic mechanisms involved in the accumulation and transport of AgNPs affect the metabolic processes of the fungus, thus inhibiting its growth. These results suggest that AgNPs possess antifungal activity and can be used in the eradication of contaminants during the in vitro culture of plant species.

Ramírez-Mosqueda, M.A., Sánchez-Segura, L., Hernández-Valladolid, S.L. et al. Influence of silver nanoparticles on a common contaminant isolated during the establishment of *Stevia rebaudiana* Bertoni culture.

PV KUMAR et al., (2018), Contamination by fungi and bacteria during the in vitro propagation of plants leads to considerable losses of biological material and precludes phytosanitary certification. The anti-microbial effect of silver nanoparticles (AgNPs) may be an alternative for the eradication of in vitro contaminants. This study evaluated the microbicidal activity of AgNPs on a recurrent fungus during the micropropagation of stevia (*Stevia rebaudiana* Bertoni). First, the fungus was isolated and identified at a molecular level by the sequencing and analysis of the

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ABDEL RAOUF et al., (2013) *Heliotropism crispum*-mediated assembly of silver nanoparticles exhibit profound antibacterial and anti-biofilm effects against multiple drug-resistant bacteria, but its stability and biocompatibility remain a hurdle in commercialization. Herein, we adopted a surface chemistry-based steric repulsion approach to investigate the colloidal stability of *H. crispum* silver nanoparticles (HC-AgNPs) and determine its application as a commercial antibacterial formulation. Two primary silver nanoparticles (AgNPs) were synthesized, i.e., HC-AgNPs and citrate-HC-AgNPs initially, which were then modulated to assemble various derivatives of AgNPs using polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) as stabilizing agents. Various parameters were assessed to investigate the morphology, colloidal physio-chemistry, and surface capping using various analytical techniques, such as UV–Vis spectrophotometer, zeta–dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Selective AgNPs

were then screened on the basis of stability for up to 8 months, heat resistance at 100 °C, and bioactivity via MTT assay using HEP-2, MCF-7, and HCEC cell lines. The UV–Vis and electron microscope results revealed that HC-AgNPs confer near-spherical and slight triangular-shaped morphology and the size of all synthesized AgNPs is in the range of 400–450 nm. All AgNPs synthesized from HC-source have characteristic FTIR peaks recorded near 2931 cm⁻¹, 1996 cm⁻¹, and 825 cm⁻¹. This study highlights promising evidence for the commercial application of HC-AgNPs as an antimicrobial agent. Additionally, it provides a methodology for modulating the surface capping of biological nanoparticles and assesses its corresponding effect on the stability. Khan, F., Iqbal, S., Khalid, N. et al. Screening and stability testing of commercially applicable *Heliotropism crispum* silver nanoparticle formulation with control over aging and biostability

SADEGHI B et al., (2015) Nanobiotechnology is an emerging field among researchers. Currently the development of plant-mediated biogenic nanoparticles is gaining much attention. It is notable that the biogenic nanoparticles particularly silver nanoparticles are therapeutically more effective than its chemically synthesized form. Thus, here in, the development of silver nanoparticles (Fj-AgNPs) using aqueous extract of ripe fruit of *Flacourtia jangomas* as a reducing agent is investigated. The developed Fj-AgNPs were additionally compared with ammine modified silver nanoparticles (Am-AgNPs), developed through chemical route using the reported approach and confirm the phyto-reduction of Ag⁺ to Ag⁰. UV–visible spectrum has exhibited a peak at 418 nm and FTIR peak profile (at 1587.6, 1386.4, and 1076 cm⁻¹ with corresponding compounds) in addition to the diffraction peak at 38°, 44°, 64°, 78° in PXRD spectrum confirmed the synthesis of Fj-AgNPs with 8.29 nm average crystallite size. Enhanced anti-bacterial effect is observed in the case of Fj-AgNPs as compared to Am-AgNps. These results refer to the idea that the use of the aqueous extract of ripe fruit of *Flacourtia jangomas* could be a good replacement of EDTA in the synthesis of silver nanoparticles. Ahmad, F., Taj, M.B., Ramzan, M. et al. One-pot synthesis and characterization of in-house engineered silver nanoparticles from *Flacourtia jangomas* fruit extract with effective antibacterial profiles.

KANNAN BADRINARAYANAN et al., (2010), Microbial contamination is a serious challenge in plant tissue culture, particularly in micropropagation of threatened and rare medicinally important plants for conservation purpose. Use of antibiotics exhibit harmful effects on plants, and continuous use makes bacteria more resistant. Also, chemicals used to control such contaminations are either toxic to the explant or have limited efficiency. Though nanobiotechnology offers an effective alternate to deal with the bacterial and fungal contamination, chemical synthesis of metal nanoparticles has limitations and found to be toxic, flammable and hard to get disposed. Green synthesis of silver nanoparticles (AgNPs) employing plant extracts, being environment friendly, cost-effective, and single step, is gaining attention as better alternative method. In this study, the green synthesised silver nanoparticles were confirmed by UV-Vis spectroscopy (462.73 nm, 0.473 Abs) and Transmission Electron microscopy (TEM). The fruit extract of *Capparis decidua* served as an environmentally benign reducing agent and the phytochemicals of the extract as non-toxic agent to stabilize the AgNP (FTIR) upholding its significance as an eco-friendly approach compared to hazardous chemicals. The nano size (1.5-15 nm) makes the green synthesized AgNPs a better antimicrobial agent allowing easy diffusion into the cells. Evaluation of decontamination as well as the survival rate of the explants was monitored using the explants (shoot tip and nodal segment) immersion in three different concentrations of AgNP solution (100, 300, and 500 mg/L) and controlled by 0.1% mercuric chloride treatment demonstrating promising decrease in decontamination. However, the survival was expedient excluding immersion in 100 mg/L for 20 or 30 min. The MS media supplementation by AgNP solution (50, 100, 300 and 500 mg/L), controlled by 70% ethanol treatment divulged the superior decontamination rate at 150 mg/L of AgNPs (90.2% for bacteria and 94.4% for fungal contamination) with 80.5% survival. The increased concentration gave 100% bacterial and 98.6% fungal decontamination but a reduced survival percent (68.5%). This work potentially showed that nanosized AgNPs could serve as an appropriate antimicrobial substitute to chemicals being innocuous to the explant regeneration. RAMIN GHahremanzadeh et al., (2019)

CHAPTER 3

AIM AND SCOPE

3.1 AIM:

To synthesis and optimize silver nano particle using biowaste and prepare Bio degradable antimicrobial food packing film to extend shelf of food

3.2 AIM AND SCOPE OF THE PRESENT INVESTIGATION:

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The use of environmentally friendly materials like biowaste extract, for the synthesis of silver nanoparticles offers numerous benefits of eco-friendly and compatibility for food packaging industries and other food science applications. By taking all these views, the present study was designed by the following objectives,

- To synthesize silver nanoparticles using the snow peas peel (biowaste).
- To analyse the synthesized Silver nanoparticles using uv- visible spectrophotometer.
- To characterize the synthesized Silver nanoparticles using TEM, FTIR and XRD
- To check its antimicrobial activity with gram -ve and +ve bacteria (food pathogen)
- To prepare biodegradable film made up gelatine and embedded with synthesised silver nanoparticle to increase its shelf life

CHAPTER 4

MATERIALS AND METHODS

4.1 METHODOLOGY:

The materials that are used for the process are:

4.1.1 EQUIPMENTS:

- Conical flasks
- Measuring cylinder
- Petri plates
- Test tubes
- Funnel
- swab
- L-rod
- Micro pipette
- Glass pipette
- Nonabsorbent cotton
- Cotton plugs
- Scraper
- Aluminum foil
- Cork borer
- Film casting tray

4.1.2 REAGENTS AND CHEMICALS:

- Sodium chloride
- Tryptone
- Yeast extract
- Agar Agar
- Hcl

- NaOH
- Silver nitrate
- Gelatin
- Glycerol

4.1.3 DEVICES:

- Centrifuge
- Incubator
- Hot air oven
- Autoclave
- Laminar air flow chamber
- UV- spectroscopy
- FTIR
- TEM
- XDR
- Magnetic stirrer
- Ph meter
- Blender

4.2 COLLECTION OF SAMPLES:

The vegetable and fruit peels of citrus x sinensis (orange peel), punica granatum (pomegranate peel), arachis hypogaea (ground nut shell), c. limetta (musambi peel), pisum sativum (pea peel) were separated from the pulp, cleaned, and shade dried for 2 weeks.



Fig (4.1) orange peel



Fig (4.2) pomegranate peel



Fig (4.3) ground nut shell



Fig (4.4) musambi peel



Fig (4.5) pea peel

4.3 PREPARATION OF EXTRACTS:

The dried vegetable and fruit peels were individually ground into fine pieces (75%cores)

The extracts were prepared by weighing 10 g of fine grounded sample mixed with 100 mL of double distilled water. Each mixture was boiled for 20 min. at 60 °C, cooled and filtered through Whatman No. 1 filter paper. The extracts are to be stored at 4 °C for further work and tests.

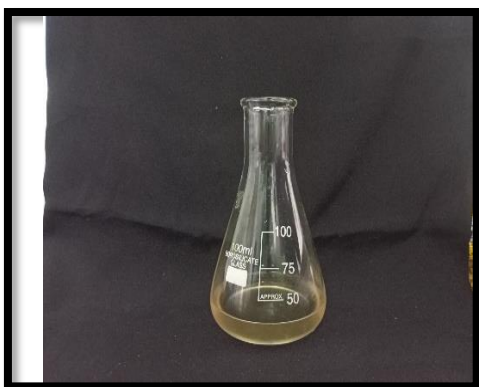


Fig (4.6) ST1 aqueous extract

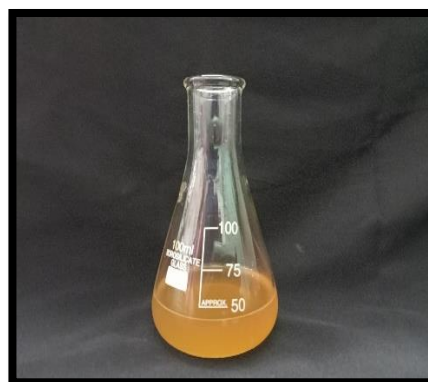


Fig (4.7) ST2 aqueous extract

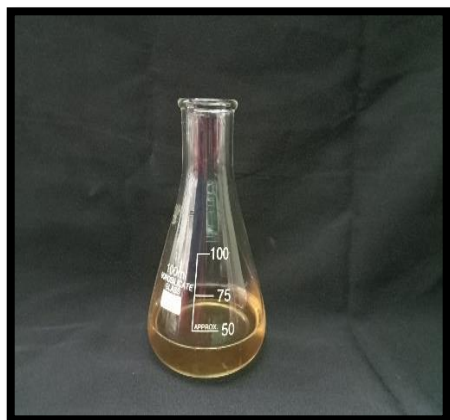


Fig (4.8) ST3 aqueous extract

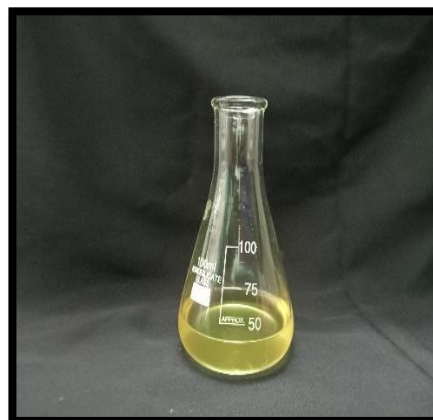


Fig (4.9) ST4 aqueous extract

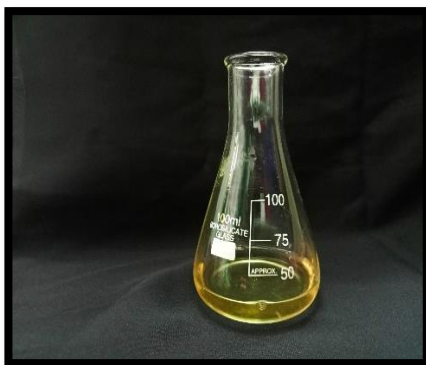


Fig (4.10) ST5 aqueous extract

4.4 SYNTHESIS OF SILVER NANOPARTICLES:

The silver nanoparticles (AgNPs) have been synthesized with the aid of mixing 10 mL of the peel extracts to ninety mL of 1mM AgNO₃ solution and stored at room temperature for 24 h in darkish condition. The exchange in visible color from a colourless solution to reddish brown was once observed. The take a look at solution of arachis hypogea (ground nut shell) and Pisum sativum (pea peel) extract exhibited reddish brown color and for this reason was once chosen for in addition study

4.5 CHARACTERIZATION OF SYNTHESIZED AGNPS:

4.5.1 UV–VIS SPECTRA AND STABILITY ANALYSIS:

The reduction of silver ions in silver nanoparticles used to be monitored via Systolic Double Beam Spectrophotometer 2202 at the wavelength vary of 300–700 nm. The stability of AgNPs used to be additionally analyzed via measuring the absorbance from the vary of 300 nm to seven-hundred nm at ordinary time intervals (24 h, forty-eight h, third day ,4th day ,5th day, tenth day, fifteenth day, twentieth day,25th day and thirtieth day).

4.5.2 TRANSMISSION ELECTRON MICROSCOPY (TEM) AND ENERGY DISPERSIVE XRAY (EDX) ANALYSIS:

The morphological shape of AgNPs such as size, shape, surface and crystal shape information had been received by using TEM. In brief, a drop of synthesized AgNPs was once positioned on carbon-coated copper grids and allowed to stand for two min, and the extra answer used to be eliminated the use of a blotting paper and allowed to dry at room temperature. The samples have been then discovered beneath TEM FEI-TECNAI G2 T-30 S-TWIN instrument.

The presence of the exclusive elemental composition of the pattern used to be studied the use of the above instrument connected with thermo EDX. The synthesized AgNPs have been remoted by way of centrifugation for 20 min. at 10,000 rpm. The pellets had been accrued and dried in the oven at 50 °C to cast off any extra water, cooled to room temperature and observed for EDX analysis.

4.5.3 X-RAY DIFFRACTION (XRD) MEASUREMENT:

The evaluation of crystallographic constructions and specific fabric identification of synthesized AgNPs segment houses have been carried out by way of XRD measurements. The powder X-ray diffractometry (SEIFERT JSO-DEBYEFLEX 2002) used to be used at an running situation of forty kV, 30 mA with Cu K α radiation at a wavelength of 1.5406 Å. The synthesized AgNPs have been lyophilized at 50 °C and the XRD sample was once scanned in the 2 θ vary from 35° to 70° with a step dimension of 0.04° per second.

4.5.4. FOURIER TRANSFORMS INFRARED (FTIR) SPECTROSCOPY:

FTIR measurements had been carried out in order to pick out the presence of a variety of practical companies in biomolecules accountable for the bio discount of Ag⁺ and capping/stabilization of silver nanoparticles. The determined extreme bands had been in contrast with fashionable values to perceive the useful groups.

4.6 ANTIBACTERIAL ACTIVITY OF SYNTHESIZED AGNPS:

The antibacterial pastime of AgNPs used to be investigated towards meals associated Gram-positive microorganism viz., *Bacillus subtilis*, *Staphylococcus aureus* and Gram-negative microorganism viz., *Escherichia coli*, *Pseudomonas aeruginosa* by way of agar nicely diffusion assay (Perez, Paul, & Bazerque, 1990).

The bacterial meals pathogens had been got from the Culture Collection Centre, IBMS, Tiramani, Chennai. The freshly cultured bacterial suspensions (100 µl) had been swabbed on Muller-Hinton agar plates aseptically and wells have been made with the assist of a sterile cork borer. The wells had been loaded with specific concentrations (15, 30, 45, 60 µg/mL) of the synthesized AgNPs. The plates had been incubated at 37 °C for 24 h and the sector of inhibition (ZOI; mm) performing round the wells was recorded.

4.7 OPTIMISATION OF AgNPs:

4.7.1 EFFECT OF PH:

pH of the reaction mixture (4 ml distilled water and 1 ml sample) was once maintained at 3, 5, 7, 9 and 11, respectively, by means of the use of (0.1 N) HCl and (0.1 N) NaOH. 1mM AgNO₃ answer 20µl is brought and saved for 24 hours incubation. The absorbance of the ensuing options was once measured spectrophotometrically.

4.7.2 THE EFFECT OF TEMPERATURE:

To find out about the impact of temperature on the synthesis of AgNPs, a ordinary pattern (4 ml Distilled water and 1 ml sample) used to be synthesized at 25 °C, 35 °C and forty five °C. 1mM AgNO₃ answer 20µl is introduced and stored for 24 hours incubation. Electronic absorption spectra of the aqueous colloidal suspensions had been recorded at every temperature range.

4.8 PREPARATION OF AGNPS INCORPORATED GELATIN FILM:

To prepare silver nanoparticles incorporated gelatin film. Firstly, gelatin should be blended with distilled water at a ratio of 2% (w/v), (i.e., one hundred ml distilled water and 2g gelatin) underneath mechanical stirring till it is absolutely dissolved (approx.1 hour) and so the film-forming answer (FFS) was once obtained. Secondly, we should add 1ml of glycerol brought into FFS as a plasticizer in movie then, 5ml of synthesized AgNPs is integrated and is allowed to spin in magnetic stirrer at 1500 rpm about 1 hour and the whole method is maintained in 20°C, the answer is then poured in film casting tray and saved undisturbed for 24 hours.

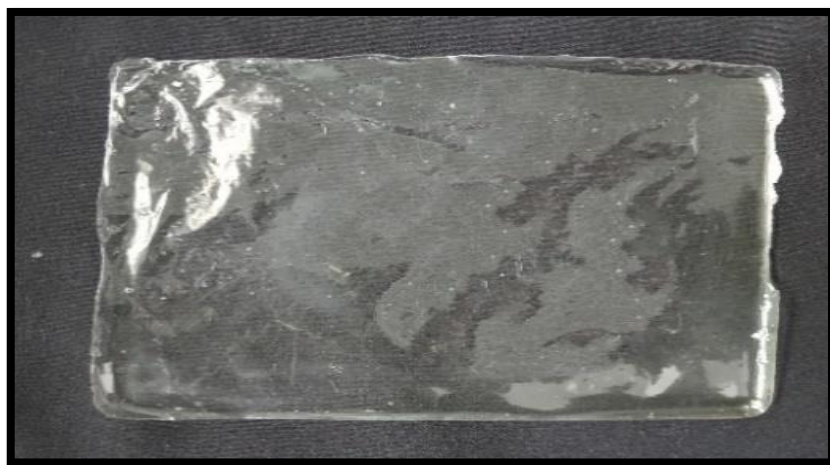


Fig (4.11) Gelatin film

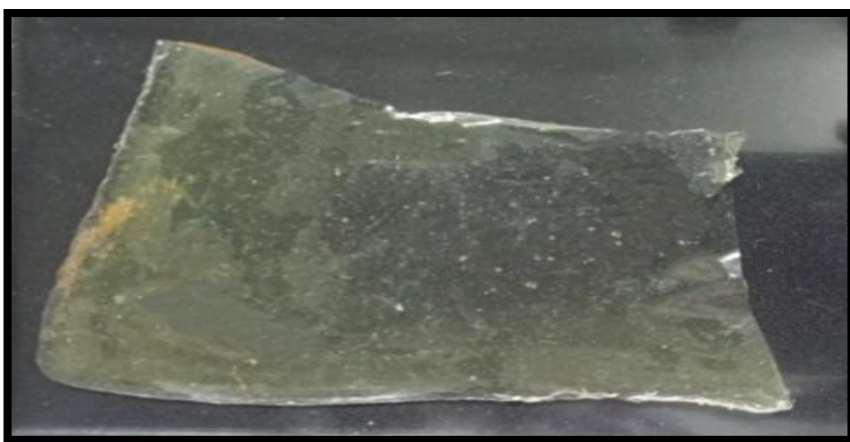


Fig (4.12) Gelatin film +AgNPs

4.9 ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF AgNPs GELATIN FILM:

The antibacterial activity of AgNPs gelatin was assessed by disk diffusion method. The bacterial culture was said to be swabbed uniformly onto the individual Mueller Hinton agar plates and with the help of sterile cotton swabs. AgNPs gelatin film and gelatin film (without AgNPs), disinfected by ultraviolet C irradiation for 1 min, were cut into 5mm discs and placed in the culture-swabbed Petri plate along with 20 μ L of positive control (Amoxicillin, 10 μ g). The plates were examined for the formation of an inhibition zone after overnight incubation at 37 °C. The diameter of the zone of inhibition (ZOI; mm) formed around the AgNPs emberded gelatin film on the plates was measured and recorded as an inhibition against the test bacterial strains.

4.10 AGNPS EMBERDED GELATIN FILM COATING ON FRUIT AND VEGETABLE:

The fruit, *Fragaria ananassa* (strawberries) and vegetable *coccinia grandis* (scarlet gourds) were surface-sterilized using 70% alcohol to remove surface contaminants like dust and dirt from fruits and thereby subsequently washed with distilled water. The surface-sterilized fruits were covered with AgNPs coated gelatin film. The coated and uncoated fruits were kept at room temperature and were periodically assessed for 10 days.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. BIOSYNTHESIS OF SILVER NANOPARTICLES:

The synthesis of AgNPs is achieved through biological reduction method where the fruit peel extract of *Pisum sativum* (pea peel) exhibited reddish brown colour and good stability as an indication of the formation of nanoparticles and the remaining four peels showed aggregation and no stability (Fig 5.1). The collective oscillation of electrons in synthesized AgNPs attributes to the colour change (Noginov et al., 2007).

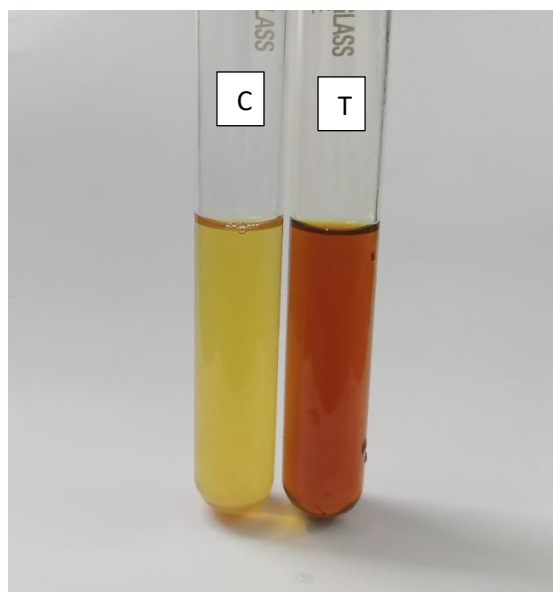


Fig (5.1) C is control and T is test solution of synthesized AgNPs from Pisum sativum

5.2. CHARACTERIZATION OF AGNPS:

5.2.1. UV-VIS SPECTRA AND STABILITY ANALYSIS:

The bioreduction of Ag^+ ions into elemental Ag^0 in the form of nanoparticles was monitored by UV-vis spectroscopy. The UV-vis spectra showed a well-defined absorbance peak at around 421.6 nm (Fig. 5.2), which is the characteristic of AgNPs

and clearly indicates the formation of AgNPs in solution. The change in coloration was observed to be intent due to the excitation of the surface plasmon vibrations in metal nanoparticles, thus the stability of the synthesized AgNPs was evaluated which revealed that the solution was stable even up to months of reaction by periodical assessment at different time durations, with no evidence of aggregation of particles (Fig.5.3). The stability results from a potential barrier that develops as a result of the competition between weak Van der Waals forces of attraction and electrostatic repulsion (Mulvaney, 1996). The UV–vis spectra also showcased no shifts in the absorbance intensity λ max hence, this is an indication of the efficiency of the AgNPs synthesis and of the unaffected particle size.

DAY 1:

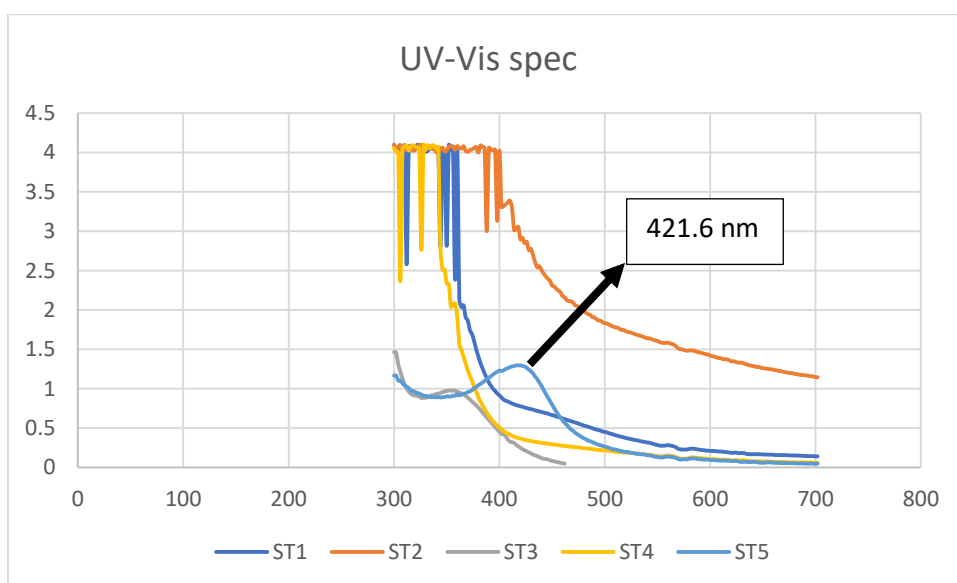


Fig (5.2) uv-vis spec readings after 24 hours

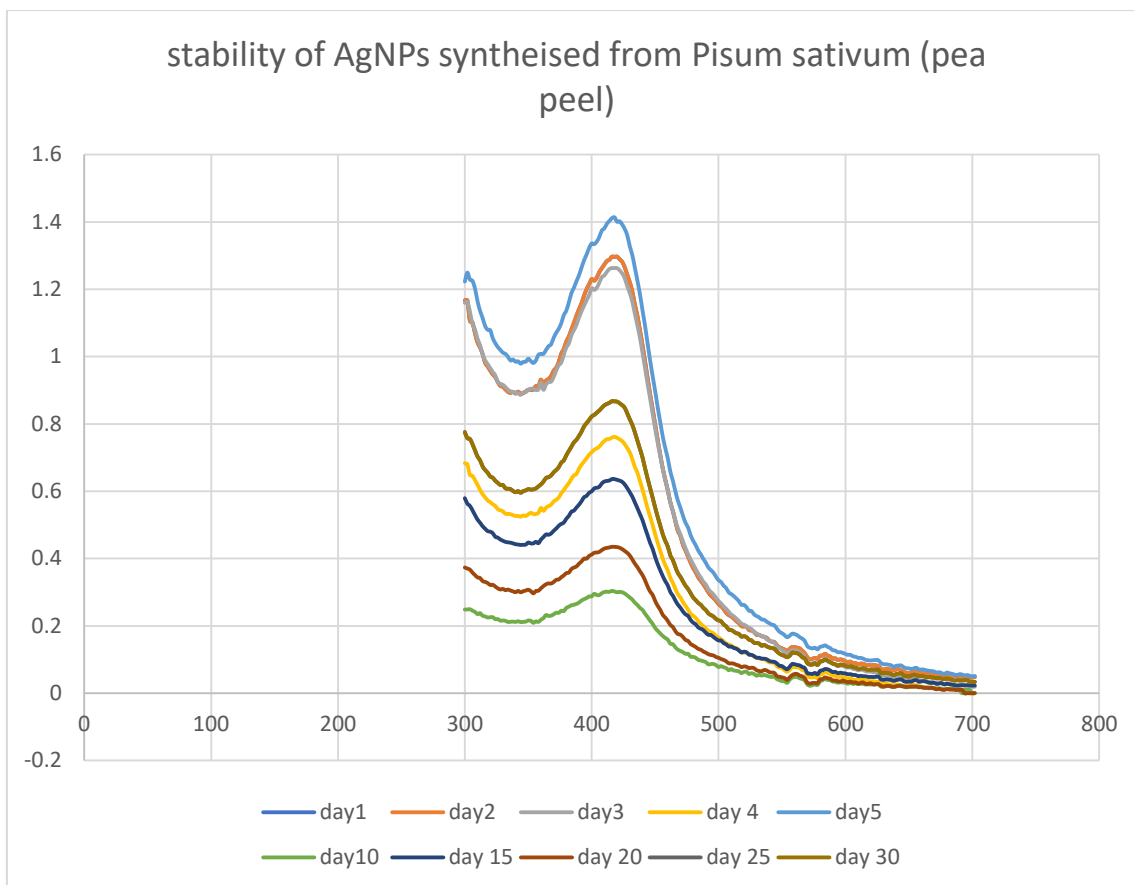


Fig (5.3) stability of AgNPs synthesised from Pisum sativum (pea peel)

5.2.2. TRANSMISSION ELECTRON MICROSCOPY (TEM) AND ENERGY DISPERSIVE XRAY (EDX) ANALYSIS :

TEM analyses confirmed the synthesis of AgNPs with spherical and quasi-spherical morphology (Fig.5.4). The TEM image showed the size of the synthesized AgNPs in the range of 10–50 nm, with an average size of 30 nm. Polydisperse, discrete AgNPs was observed in the micrograph (Fig.5.5).

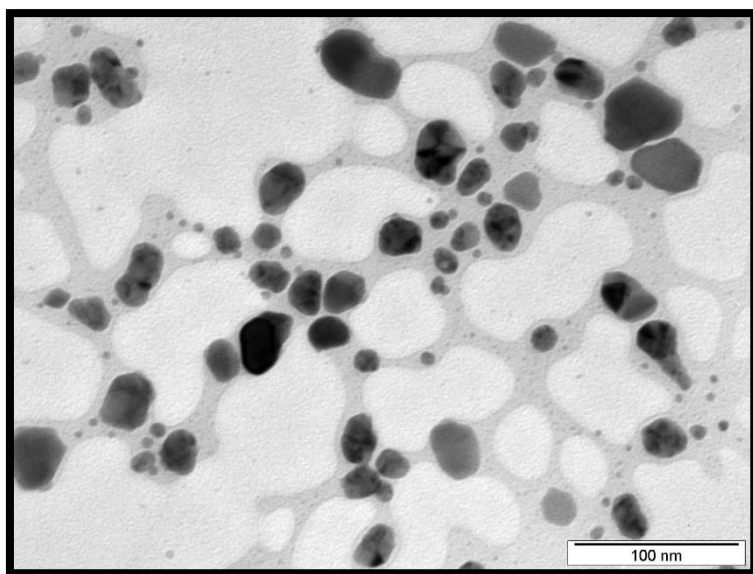


Fig (5.4) TEM image at 100 nm

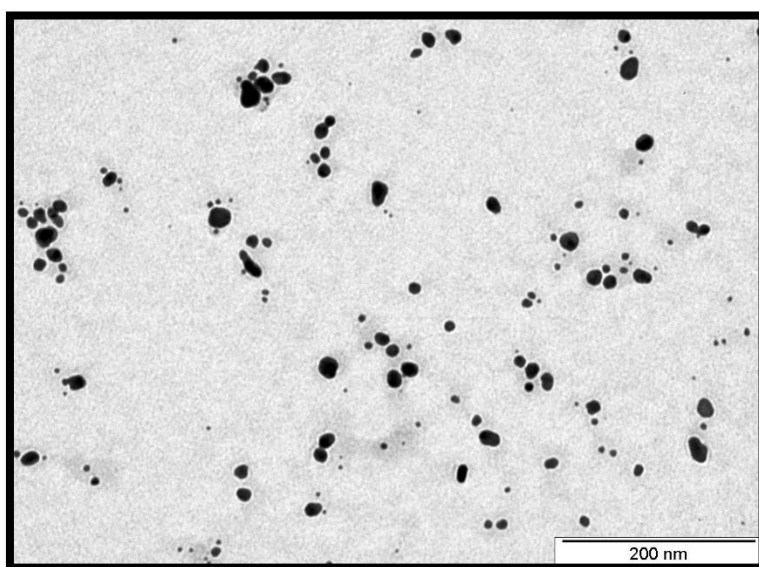


Fig (5.5) TEM image at 200 nm

The EDX spectrum of AgNPs showed strong signals for the atoms in the solution at 3 keV, typical for the absorption of metallic silver nanocrystalline due to surface plasmon resonance (Fig.5.6). This distinctively demonstrates the reduction of the silver ions in the silver nitrate to the elemental silver. The scanning range of binding energies depicts no peak of impurity, thus indicating the high purity of AgNPs.

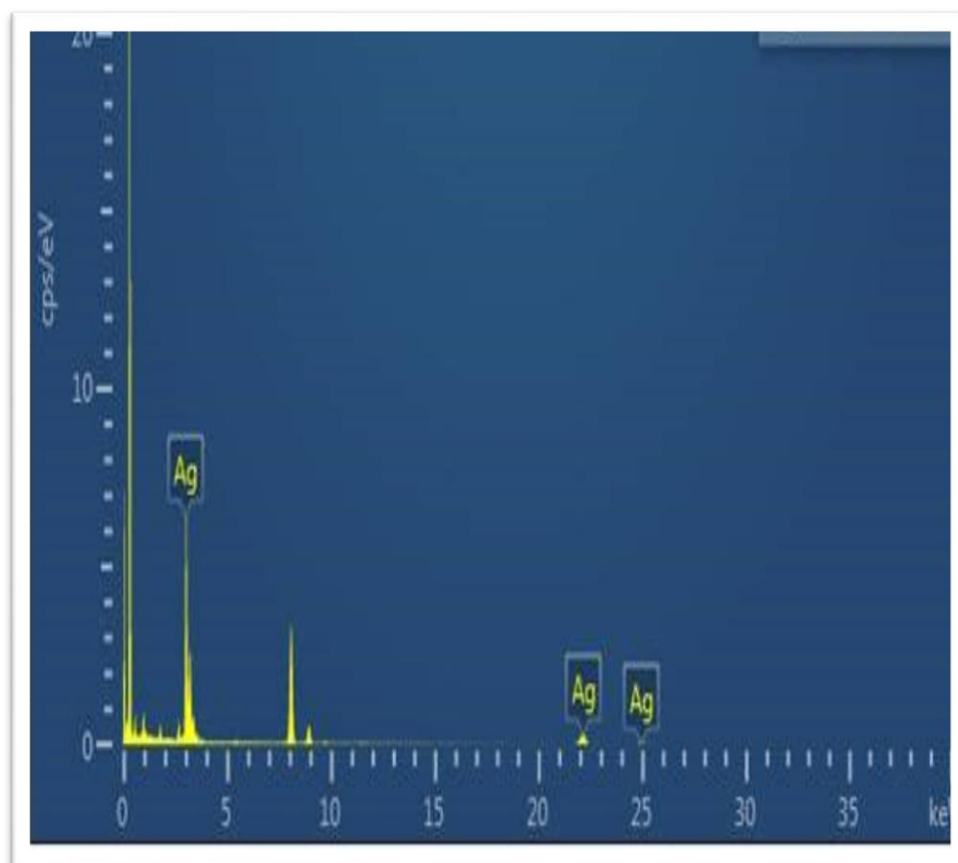


Fig (5.6) EDX spectrum

5.2.3. X-ray diffraction (XRD):

The crystalline nature of AgNPs was established from XRD analysis. The intensity of peaks displays the excessive degree of crystallinity of the AgNPs. The three diffraction peaks found at 38.06° , 44.23° , and 67.43° in the 2θ range can be listed to the (111), (200), (220) reflection planes of the face-centered cubic structure of metallic silver nanoparticles (JCPDS range 04-0783, 1991). The same pure silver nanocrystals and XRD noise of AgNPs may be attributed to the complex biomolecules from *Pisum sativum* (pea peel) extract.

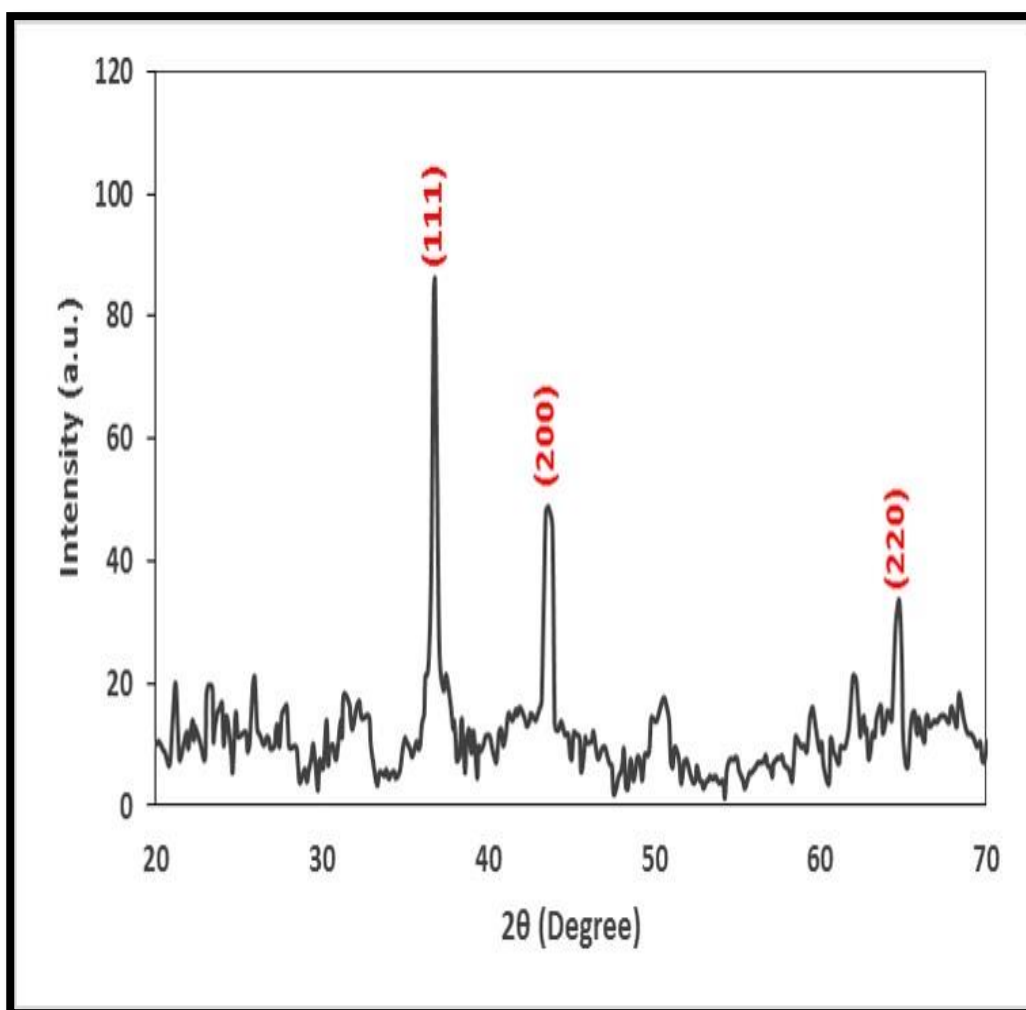


Fig (5.7) X-ray diffraction

5.2.4. FOURIER TRANSFORMS INFRARED (FTIR) SPECTROSCOPY:

FTIR spectrum shows absorption bands at 3290.56, 1635.64, 1408.04 and 1278.81 cm^{-1} indicating the presence of capping agent with the nanoparticles (Fig 5.8).

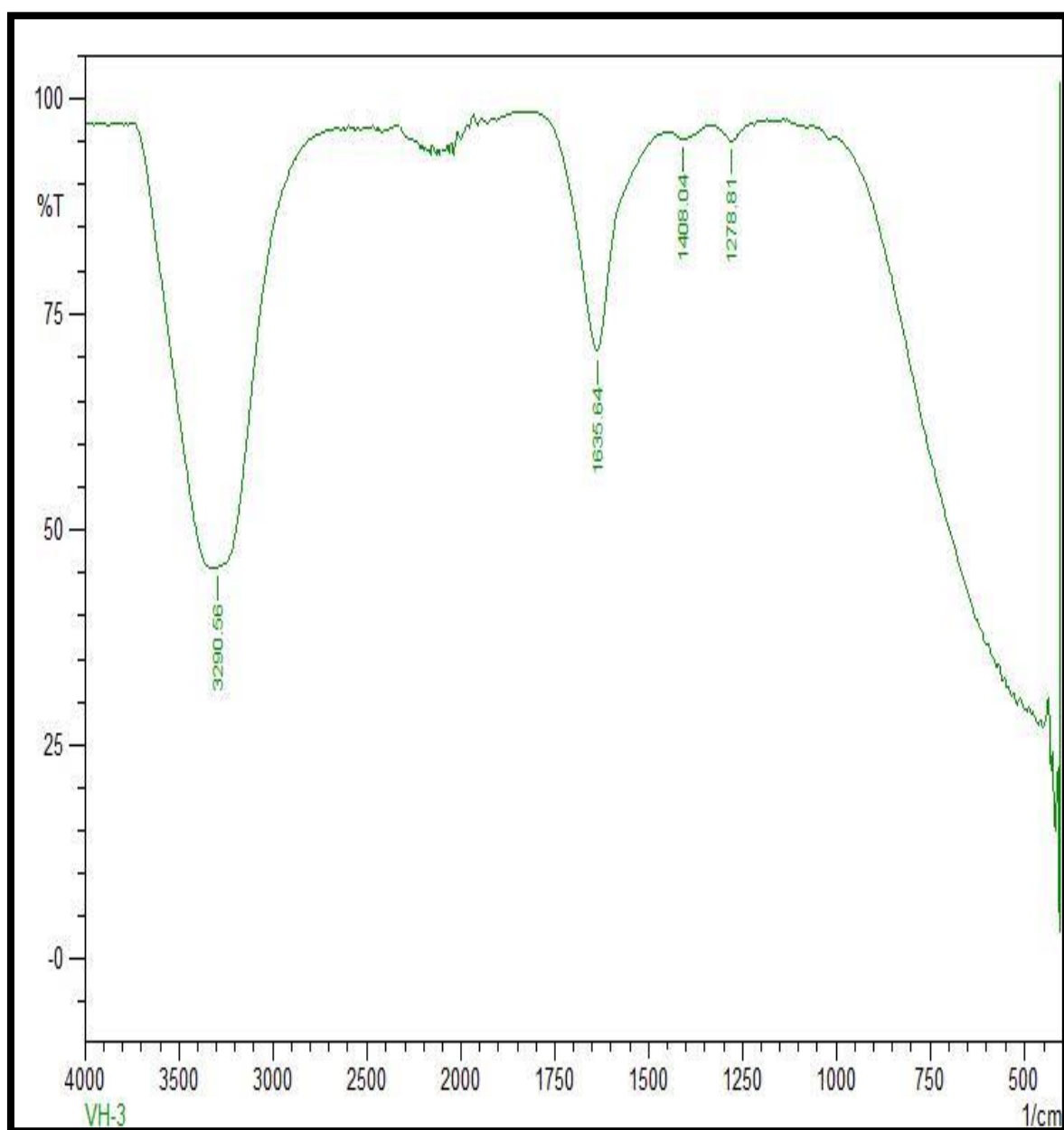


Fig (5.8) FTIR spectroscopy

Obtained wave length	Wave length	Bond type	Functional group	Stretching /bending	Functional group
3290.56 cm^{-1}	3300-2500	strong	O-H	stretching	Carboxylic acid
1635.64 cm^{-1}	1662-1626	medium	C=C	Stretching	alkene
1408.04 cm^{-1}	1410-1308	strong	S=O	stretching	Sulfonyl chloride
1278.81 cm^{-1}	1310-1250	Strong	C-O	stretching	Aromatic ester

Table (5.1) FTIR interpretation

5.3 OPTIMISATION OF AgNPs:

5.3.1 EFFECT OF PH:

pH of the reaction mixture was maintained at 3, 5, 7, 9 and 11 respectively. The absorbance of the resulting solutions was measured spectrophotometrically and observed the peak at 4.18nm with maximum absorbance as 1.12.



Fig (5.9) ph optimization sample

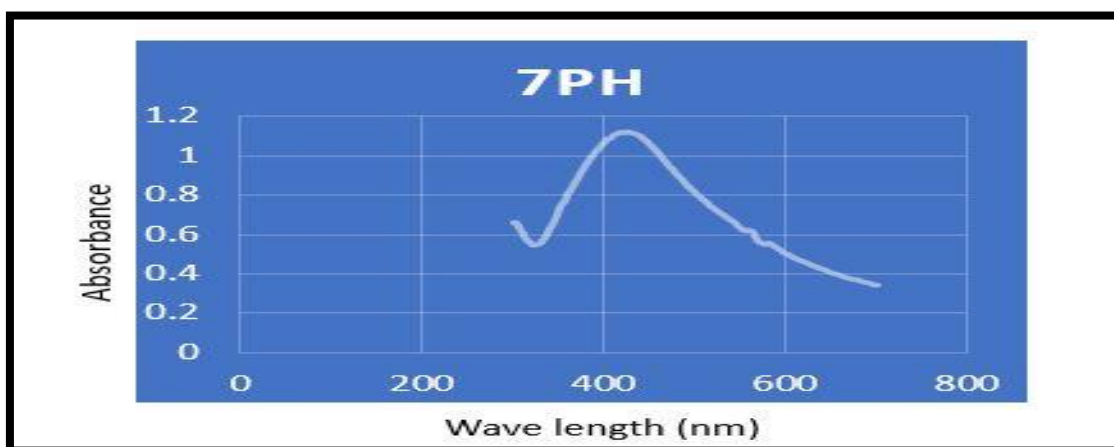


Fig (5.10) graph of ph optimization

5.3.2 THE EFFECT OF TEMPERATURE:

To learn about the impact of temperature on the synthesis of AgNPs, a typical sample was synthesized at 20 °C, 36 °C ,48°C and 60 °C. Electronic absorption spectra of the aqueous colloidal suspensions have been recorded at every temperature range. and located the optimum temperature is 36°C with the aid of the maximum absorbance of 1.28 at 430 nm

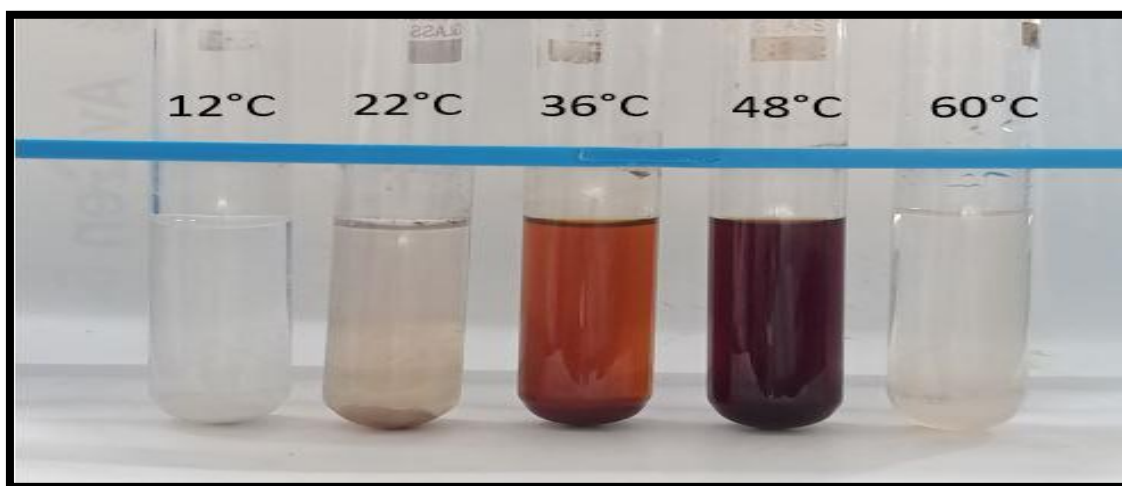


Fig (5.12) graph of temp optimization

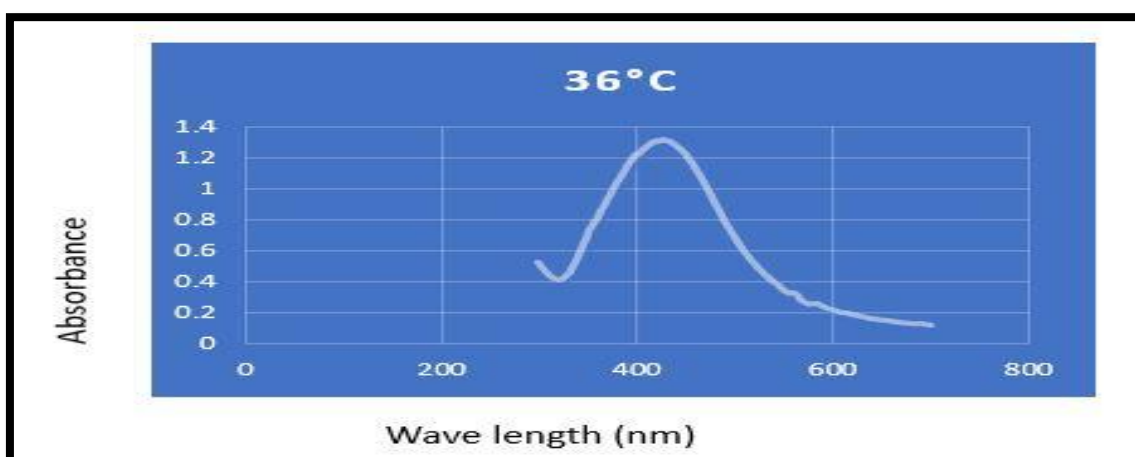


Fig (5.11) temperature optimization sample

5.4. ANTIBACTERIAL ACTIVITY OF SYNTHESIZED AGNPS:

Bacterial infections of extraordinary sorts of food pose a mission in the prevention of meals from decaying. The formation of the inhibition area is associated to the diffusion of the antibacterial agent and the increase fee of the microorganism, the place the presence of halos with diameters $\geq 10\text{mm}$ suggests an inhibitory activity. The synthesized AgNPs used to be observed to be pretty lively in opposition to examined meals pathogens, with various zones of inhibition (Fig.5.14). The effects printed that all the take a look at micro organism had been sensitive to growing attention of AgNPs (Table 1; $P < 0.05$), wherein, the Gram-positive micro organism was once located to be extra sensitive to AgNPs in contrast to Gram-negative bacteria. While AgNPs action mechanism is nevertheless now not thoroughly known, it has been stated that AgNPs on interplay with the outer membrane of bacteria, consequences in structural changes, which in flip might also lead to an extend in membrane permeability and leakage of intracellular ingredients and incite extreme damage, ensuing in cellphone death. The distinction in bacterial susceptibility might also be due to structural factors in the telephone membrane of Gram nice and Gram-negative micro organism (Sondi & Salopek-Sondi, 2004; Vadillo-Rodriguez, Schooling, & Dutcher, 2009). The lipopolysaccharides (LPS), proteins, lipids of Gram-negative micro organism mobile wall ensures extra advantageous defence in opposition to biocides on contrast to Gram-positive micro organism the place phone wall does now not comprise outer membrane of LPS, in addition, the dynamic lipopolysaccharide O-side chains unexpectedly strikes and can also disable the formation of a metal ion salt bridge and forestall the antibiotic impact when AgNPs are no longer current in enough concentration, as a consequence main to lesser antibacterial activity on Gram-negative micro organism than Gram-positive micro organism (Langley&Beveridge, 1999; Maneerung, Tokura, & Rujiravanit, 2008).

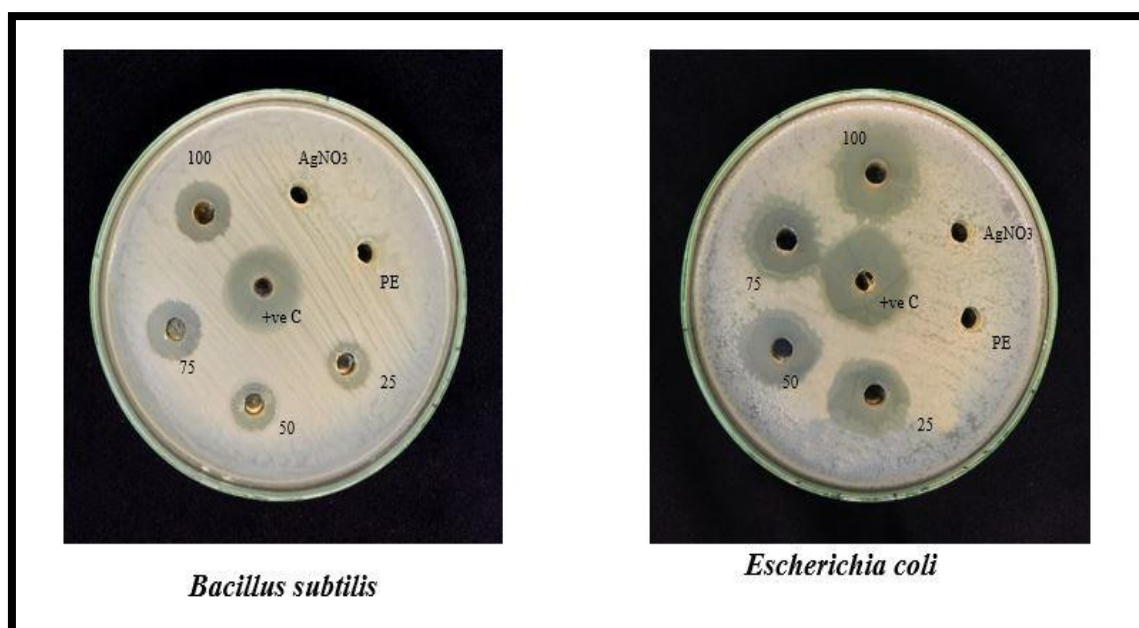


Fig (5.13) Anti-bacterial activity of AgNPs

S. No.c	Name of microorganism	Zone of inhibition (mm in diameter)						
		Positive control (Amoxicillin)	Plant Extract (PE)	Silver Nitrate (AgNO ₃)	Silver Nanoparticles (AgNPs; μ L)			
					25	50	75	100
1	<i>B. subtilis</i>	22	-	-	08	09	12	16
2	<i>E. coli</i>	26	-	-	21	23	24	25

Table (5.2) ZOI (zone of Inhibition) of synthesisations

5.5 ANTIBACTERIAL ACTIVITY OF AgNPs COATED GELATIN FILM:

The assessment of the antibacterial activity of AgNPs coated gelatin film showed significant inhibitory activity against all the tested foodborne microorganisms (Fig.5.15). A significant difference in the antibacterial activity of AgNPs embedded gelatin film was observed against test pathogenic bacteria (Table, $P < 0.05$). The AgNPs coated gelatine film showed superior activity (ZOI) against *e. coli* with an average diametric of halos equal to 19 mm. Similar adequate results have been said reported earlier by Zhang et al. (2016) that showed superior bacterial inhibition against the Gram-positive bacteria. The gelatine film (without AgNPs) showed no antibacterial activity. This confirms that the antibacterial ability of AgNPs coated gelatine film depends on the presence of AgNPs on the surface of the film. According to Moura, Mattoso, and Zucolotto (2012)), the smallest dimension particles with the highest specific area can better disperse into the polymers, which promotes the release of the silver ions more efficiently to the culture medium. The results substantiate that AgNPs emberded gelatine film possess prominent antibacterial activity and could protect the foods from microbial infections.

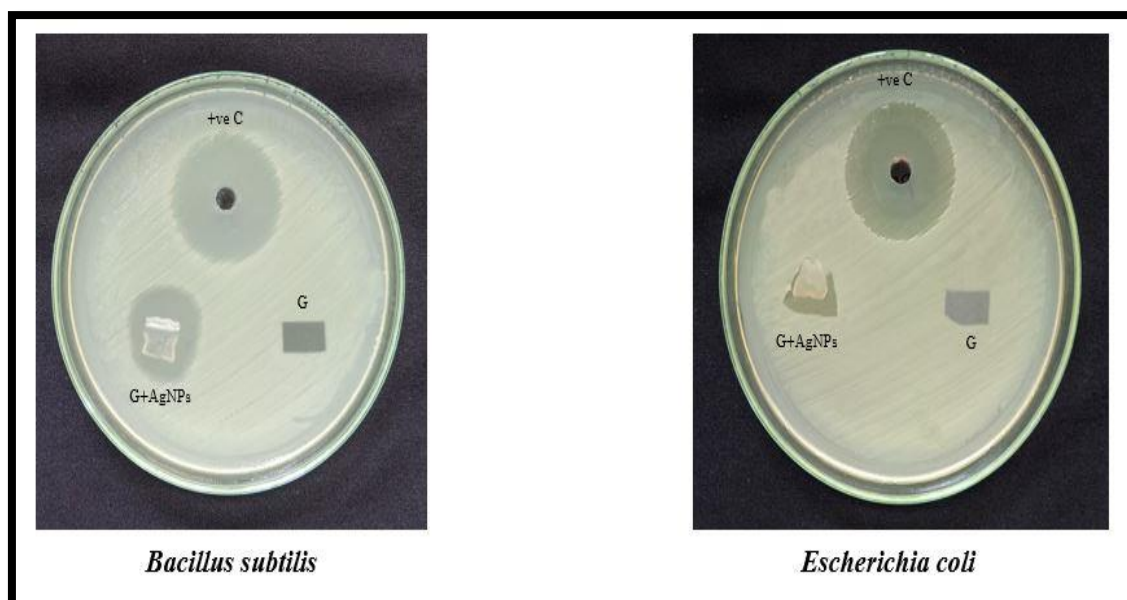


Fig (5.14) Anti-bacterial activity of AgNPs coated Gelatin film

S.No	Name of microorganism	Zone of inhibition (mm in diameter)		
		Positive control (Amoxicillin)	Gelatin film (G)	Gelatin and Silver nanoparticles (G+AgNPs)
1	<i>B. subtilis</i>	26	-	14
2	<i>E. coli</i>	28	-	19

Table (5.2) ZOI (zone of Inhibition) of synthesised AgNPs + gelatin film

5.6. PRESERVATION EFFECT OF AgNPs EMBEDDED GELATINE FILM ON FRUIT AND VEGETABLE:

The preservation effect of uncoated (control) and AgNPs embedded gelatine film coated strawberries and scarlet gourds are represented in Fig. (5.16). the strawberry is a very delicate and highly perishable fruit having very short postharvest lives and gets decayed by microbial infection within two to three days. In the present study, AgNPs embedded gelatine film coated strawberries and scarlet gourds were acceptable and not decayed up to 10 days of storage, as judged by the colour, appearance, and texture compared to uncoated control strawberries and scarlet gourds, and thus revealing an increased shelf life of the fruits. No significant or varied changes in weight were observed in the coated fruits and vegetables. There was no overall acceptance for uncoated control fruits after the 3rd day of storage, due to the gradual increase in the microbial infection on scarlet gourds and strawberries, leading to their decaying process. The uncoated scarlet gourds showed a change in texture from smooth to wrinkled with the formation of rot on the peel and the uncoated strawberries presented more amount of mold and yeast growth. Thus, the AgNPs embedded gelatine film exhibited an enhanced shelf life of the fruit and vegetable compared to the earlier reported studies of Gudadhe et al. (2014); Liu, Wang, Lan,

and Qin (2017)); Sangsuwan, Pongsapakworawat, Bangmo, and Sutthasupa (2016)); Senna, Al- Shamrani, and Al-Arifi (2014)); Valenzuela, Tapia, López, Escalona, and Abugoch (2015)), and contributes to research in cost-effective and biodegradable nanocomposite antimicrobial packaging to overcome the transportation and storage problem of tropical fruit export commodities.

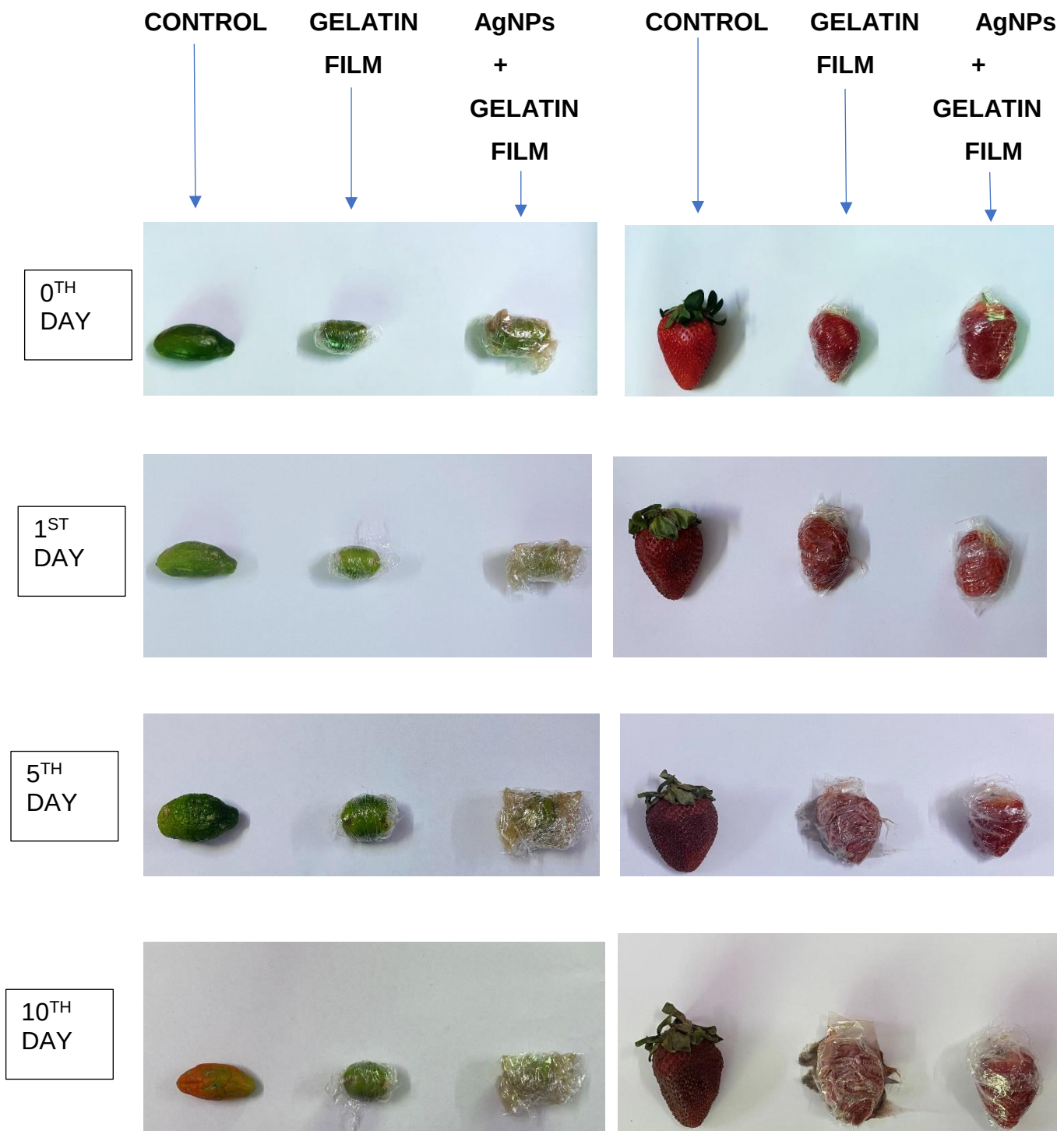


Fig (5.15) strawberry and scarlet gourd in preservation film

CHAPTER 6

SUMMARY AND CONCLUSIONS

Silver nanoparticles based antimicrobial packaging is an innovative concept for active food packaging which plays an important role in extending the shelf life of foods and reducing the risk of pathogens infections. The present study exhibits that the peel extract of *pisum sativum* (pea peel) possesses the capacity to synthesize silver nanoparticles of significant bactericidal activity. The in vitro analysis revealed that AgNPs embedded gelatin film exhibited the highest antimicrobial activities, which was confirmed by the application of the AgNPs embedded gelatin film for the shelf-life extension in scarlet gourd and strawberries.

The active AgNPs embedded gelatin film enabled the inhibition of the microorganisms in fruits for a period of 10 days of storage at room temperature without the use of a modified atmosphere, which was three times more than the control fruits (uncoated). The preparation of AgNPs incorporated gelatin film and their application for food preservation proved to be a novel method and an efficient mode for increasing the shelf life of fruits and vegetables. It's a new approach to utilize the nanobiotechnology field for food preservation.

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