

**Natural saccharides mediated biogenic synthesis of
noble metal nanoparticles and explore its application in
biomedical sciences**

A Thesis Submitted

In partial fulfillment of the requirements for the award of degree of

Masters of Technology

In

Biotechnology



By

Anmol Bansal (131576)

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

Waknaghat, Solan (H.P) India

TABLE OF CONTENTS

S. No.	Topics	Page No.
1)	Certificate of Originality	3
2)	Acknowledgement	4
3)	Preamble	5
5)	Chapter 1: Introduction	1-7
6)	Proposed Work Plan	8
7)	Chapter 2: Literature Survey	9-14
8)	Material & Methods	15
9)	Chapter 3: Experimental Approach	16- 19
10)	Chapter 4: Results	20-29
11)	Conclusion	30
12)	References	31-32

List of Figures

Fig. no.	Caption	Page no.
1	Methodologies utilized as a part of developing Nano-biomaterials	4
4.1	Image shows initial colour of the mixtures in the four conical flask	21
4.2	Colour of samples after mixing and keeping the solutions in sunlight	21
4.2.1	The absorbance spectrum of glucose silver nanoparticles showing maximum absorbance near 450nm	22
4.2.2	The absorbance spectrum of sucrose silver nanoparticles showing maximum absorbance near 450nm	22
4.2.3	The absorbance spectrum of ribose silver nanoparticles showing maximum absorbance near 450nm	23
4.2.4	The absorbance spectrum of fructose silver nanoparticles showing maximum absorbance near 450nm	23
4.3.1	SEM image showing surface morphology of fructose silver nanoparticles	24
4.3.2	SEM image showing surface morphology of sucrose silver nanoparticles	24
4.3.3	SEM image showing surface morphology of glucose silver nanoparticles	25
4.3.4	SEM image showing surface morphology of ribose silver nanoparticles	25
4.4.1	EDX curve of fructose Ag-nanoparticles showing the presence of Ag and other elements (C and O)	26
4.4.2	EDX curve of fructose Ag-nanoparticles showing the presence of Ag and other elements (C and O)	26
4.4.3	EDX curve of fructose Ag-nanoparticles showing the presence of Ag and other elements (C and O)	27
4.4.4	EDX curve of fructose Ag-nanoparticles showing the presence of Ag and other elements (C and O)	27
4.5.1	The disk D1 shows Inhibition Zone for sugar solution where negative control shows no result	28
4.5.2	The disk D2 shows Inhibition Zone for sugar solution where negative control shows no result	28
4.6.1	Shows the "catalytic capacity of AgNP's	29

CERTIFICATE OF ORIGINALITY

This is to certify that the work submitted in this report entitled: “**Natural saccharides mediated biogenic synthesis of noble metal nanoparticles and explore its application in biomedical sciences**” submitted by **Anmol Bansal** in partial fulfillment of the requirements for the award of degree of Masters of Technology in Biotechnology, of Jaypee University of Information Technology, Solan, has been carried out under the supervision of Dr. Abhishek Chaudhary. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Dr. Abhishek Chaudhary

Assistant Professor

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

Waknaghat, Solan, H.P-173234 India

Email: abhishek.chaudhary@juit.ac.in

Date:

ACKNOWLEDGEMENT

First and foremost, we are grateful to the God for the good health and wellbeing that were necessary to complete this research.

I wish to express my sincere thanks to my respected Guide **Dr. Abhishek Chaudhary**, Assistant Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, for his encouragement and providing me with all the necessary facilities for the research. I place on record, my sincere thank you to **HOD Associate Prof (Dr.) Sudhir Kumar**, for his advice and all necessary facilities to accomplish this endeavor.

I take this opportunity to express gratitude to all of the Department faculty members for their help and support. I would also like to thank my parents for their encouragement, support and attention.

I also place on record, my sense of gratitude to one and all, who directly or indirectly, have lent their hand in this venture.

Anmol Bansal

PREAMBLE

Research and advancements in technology are prompting acknowledgment of this innovation in existence because it keeps on giving arrangements and different choices to mechanical, ecological, and prosperity challenges. Nanostructures are the matter of enthusiasm for all utilizations of technology whereby form and size of the nanoparticles (NPs) decide their trademark property. Thanks to the developing interest for various Nanoparticles, it's necessary to make uniting techniques that are financially savvy and condition benevolent. Most of these techniques utilized for nanoparticle combination depend on physical and concoction ways that often embody dangerous and risky chemicals. Besides, the actual necessity for size and state of nanoparticles cannot be met with the physico-substance techniques. During this regard, natural techniques as well as microorganisms or plant removes are a lot of viable. The connexion of the standards of inexperienced science to technology toward the mix of "green" nanoparticles could be a gift necessity.

CHAPTER 1

INTRODUCTION

Researchers right now discuss the future results of nanotechnology. Nanotechnology may be able to make different new materials and gadgets with a titanic degree of employments, for example, in nanomedicine, nanoelectronics, biomaterials centrality creation, and buyer things. Then again, nanotechnology raises a basic number of a vague issues from any new progression, including worries over the unsafe quality and normal effect of nanomaterials, and their potential consequences for general money related issues, and moreover speculation about different doomsday conditions[9]. These burdens have impelled an open thought among help social events and governments on whether extraordinary control of nanotechnology is protected. Research and advancements in Nanobiotechnology are exceptional purposes behind yielding this advancement in regular existence since it has the tendency to be answerable to mechanical, health and natural challenges. Nanoparticles are the units of eagerness for its usage in all the aspects of Nano-biotechnology where crucial properties of nanoparticles are chosen on the basis of their conditions and size. The experimentation studies rely upon the changes in protein in context to different nanoparticles. Any kind of changes in the protein structure can lead to differential ailment and diseases like neurodegenerative disorders, for which there has been no treatment found till today this is because of the restriction in the blood brain barrier which can be cured or certainly overcome by the use of nanoparticles. So, people have developed keen interest in developing different formulations of nanoparticles, using different procedures and methodologies. This particular experimentation is primarily focussed on creating physico-chemical mix combined with the advancements of protein-nanoparticle complex, also its impact on the physical properties of proteins that are absorbed and there effects on the functions at cellular level. Association of nanoparticles with chemical substances has risky effects, therefore, association with green amalgam has attracted the researchers as it has environmental benefits [6]. Bovine Serum Albumin was mixed with gold and zinc to study the impact of nanostructures on proteins, the test was performed at varied concentrations. Vitality, compliances and adsorption were analysed using spectrophotometer, Dynamic light dispersing, indirect dichroism and fluorescence

quenching techniques [11]. The results from fluorescence quenching tells about the association whether end on or side on of BSA to nanoparticles.

Applications

A list of some of the applications of nanomaterials to biology or medicine is given below:

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

Proteins and nanoparticles can be differentiated from each other on the basis of size thus, makes it fit for labelling or bio-labelling. However, size is just one parameter to distinguish between the two, but its definite size is enough to make the nanoparticles a useful tool to be used as biological materials. The main considerations to be taken is to make the nanoparticles and use it in its most natural form focusing on its organic part preferably [4]. Biological coatings may include biopolymers like collagen, antibodies, monolayers that increases the compatibility of nanoparticles. Likewise, recognition systems like optical systems are boundless in biological research, nanoparticles works in multidimensional form either through fluorescence or by changing their optical properties [6].

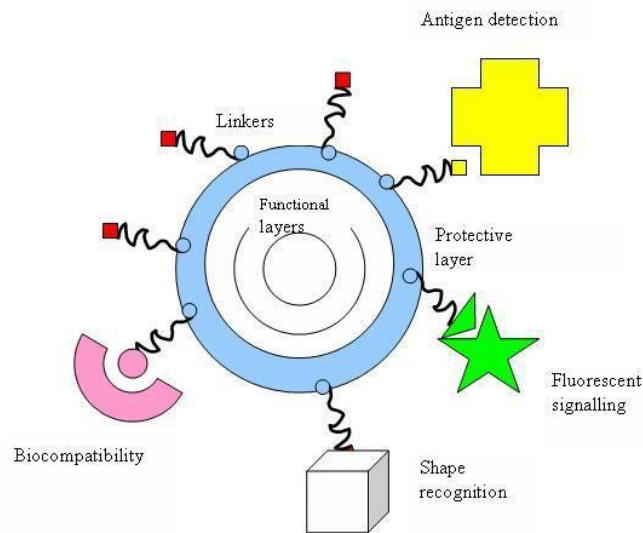


Fig 1: Procedure for developing nano-materials

The heart of any nano-biomaterial is the nanoparticle. It can either be polymeric or inorganic and has a beneficial role as sub-atomic surface. The general shape tends to be in a circular form like plate like or barrel shaped or any other related shapes are possible. The properties like size is an important aspect when it comes to the structure of the pore [14]. Estimation of normal molecule in a form of control when the circulation of sizes is limited, it permits the fluorescence efficiently and the light produced is limited at wide range of wavelengths. This has an advantage in making biomarkers that are desired. These can have many functions as well as many layers. Example of combining luminescent and magnetic layers can be recognized efficiently and in a controlled manner [12]. Few atoms or monolayers made of inorganic material can be used to secure the core materials, and can make it biocompatible and environment friendly. In order to enhance the functional ability the monolayers can be linked to the additional layer of the linker units [10]. The linker units have to end functions that is to connect the linker itself with the surface of the nanoparticles and the other is to knot down the moieties so that they are biocompatible.

Gold, Silver are the forms of nanoparticles that differ in their structural composition. New and modified tools in nanotechnology has enabled the refinement of the technique in context to its utility and applicability.

Synthesis of nanoparticles can be done through top-down approach or bottom-up approach.

In **Top-down approach** the bulk material undergoes breakdown to form particles in the size range on nanometers. Whereas, **Bottom-up approach** involves materials miniaturization and then the components undergo the process of self-assembly which results in the formation of nanoparticles. Apart from these methodologies there are different ways of producing nanoparticles like biological, chemical and physical methods [5]. Generally used method for production is through **chemical synthesis**, however this process is hazardous to the health of the living system as well as the environmental surroundings, but this can be overcome by preferably using the method called green synthesis. This method of synthesis is environment friendly as it makes the use of viruses, plants, microorganisms and their released side products such as lipids and proteins [7]. Leaves, flowers, stems, roots parts can be used when plant is used as a source of green synthesis. Though, a serious concern arises when using plant as a material for synthesis of nanoparticles because of their minimum availability in nature, therefore, plants with such importance and increased availability can be used for the same like, *Catharanthus roseus* having medicinal properties can be used in the curing of diabetes, diarrhea, high blood pressure [11] and therefore, can produce nanostructures by the use of bio based methods. *C. roseus* can be used in providing relief from pain during the treatment of cancer, and causes restriction in diabetes and leukemia that are severe forms of diseases and is primarily cultivated to extract the alkaloids for medicinal purposes [13]. Because of certain features of Nanoparticles they have attracted many researchers to extract it further for its applications:

1. It provides better surface-to-volume ratio

2. It has better Biocompatibility
3. Delivery of drugs is controlled
4. When ligands are attached to site specific targets it can be used industries.
5. Has applications in therapeutics in forms of drugs with less side effects.

Various techniques can be used to characterize nanoparticles on the basis of morphology, size to analyze the functional groups present and also to study the interactions between proteins and nanoparticles. The techniques involved are:

1. Adsorption spectroscopy or UV- spectroscopy between a particular range of UV and Visible and it analyses whether the product has been formed or not and what is their size. UV- spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the UV-Vis spectral region. The range of UV region is 190 nm- 380 nm and that of visible region is 380 nm- 750 nm so, the range of UV-Vis is 190 nm- 380 nm. It is used to determine whether the particles have formed or not and also size of particles. It is based on Beer- Lambert's law which says absorbance is directly proportional to concentration of the sample and the path length: $A \propto CL$.
2. The other principle on what the nanoparticles depend is Dynamic Light Scattering, it measures the random changes in the light intensity using a suspension solution and also identifies the profile of size distribution.

3. A technique called Fourier Transform Infrared Spectroscopy is used to obtain infrared spectrum of absorption or emission of solid, liquid or gas. It is based on the principle that most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000 – 600 cm^{-1} . It is used to determine the functional group present on the nanoparticles.
4. Transmission Electron Microscopy (TEM): It is a technique in which a beam of electron is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through it. It is based on Illumination - Source is a beam of high velocity electrons accelerated under vacuum, focused by condenser lens (electromagnetic bending of electron beam) onto specimen. It measures the size of nanoparticles, and their morphology.
5. A techniques involving polarized light is used called as Circular dichroism. Changes in secondary structure of proteins is measured through this technique depending on chiral properties of proteins.
6. A type of fluorescence spectroscopy that analyzes fluorescence from a sample. It comprises of using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and makes them to emit light; typically, but not necessarily, visible light. It used is to measure the change in spectra due to the interaction of protein with the nanoparticles.
7. Zeta Potential: The potential difference existing between the surfaces of a solid particle immersed in a conducting liquid (e.g. water) and the bulk of the liquid. It is an important

parameter that is related to nanoparticles stability or aggregation in dispersion, and can have significant implications on product performance.

8. A fast analytical technique called X-ray powder Diffraction is primarily used for phase identification of a crystalline material and can give information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined. It is based on the interaction of the incident rays with the sample produces interference when conditions satisfy Bragg's Law ($n\lambda = 2d \sin \theta$). It gives crystal structure [7]

PROPOSED WORK PLAN:

SYNTHESIS OF NANOPARTICLES



OPTIMIZATION OF NANOPARTICLES



CHARACTERISATION OF NANOPARTICLES



APPLICATION- BIOMEDICAL SCIENCE

CHAPTER 2

LITERATURE REVIEW

The train of nanotechnology is quickly evolving as an interdisciplinary science, interfacing synthetic, medicinal, ecological and physical sciences not abandoning differing designing fields, with heap of utilizations in the improvement of biosensors and biomedical gadgets, elective vitality age and natural restoration [1]. Various nanostructures, for example, thin movies, nanospheres, nanorods, and an assortment of nanoparticles (both metallic and non-metallic) are progressively adding to a few innovative applications.

Mechanical unrest in the twentieth century has prompted the gathering of gigantic amounts of hurtful modern squanders bringing about various medical issues [3]. Since its coming, nanotechnology has been indicating potential in numerous far-fetched regions making them implemental as Nanoparticles. Regular methods for nanoparticle combination fuse physical or synthetic courses, including harmful chemicals as substance forerunners to change large scale or mass materials into the nanoparticulate frames [2]. One of the objects of this exploration is to decrease the utilization of dangerous methodology through elective course on NP synthesis. Application of green science standards to the field of nanotechnology was presented by scientists about a decade ago. Eco-accommodating, green nanotechnological forms are accepted to have the capacity to deliver new items by using eco-friendly materials [4]. Such forms have included plant metabolites and plant concentrates and results of natural macromolecules, for example, nucleic acids, peptides or proteins, starches, and lipids as well. It is currently well-demonstrated that the organic course for combination of nanoparticles spares vitality and makes similarly less measure of harmful waste.

Green nanotechnology supports principal as well as objective situated research in both the scholastic and modern fields for the outline and advancement of Green Nanoparticles (GNPs) [6] [7]. Green nanoparticles have just been utilized as a part of the plan of brilliant electronic gadgets, life-sparing nano-pharmaceuticals, and in substitute environmentally friendly power vitality generation gadgets also.

In this analysis, we have antimicrobial NP [9] in a manner that can increase the impact of bioplastics for the use of green nanoparticles, reflected in a variety of ways to connect. In addition, we develop and improve the use of antibiotics in medicine and the promotion of antimicrobial silver nanoparticles and discuss measures and nanotechnology, green building is limited.

NP management, topology and physical properties necessary to take on the role of aircraft size, and email. NP scientists silver and most of the great tribulation [11].

Because of the episode of multi-medicate safe microorganisms to regular antimicrobial medications accessible in the market, the focal point of the scientists over the globe has moved to the improvement of novel antimicrobial specialists. Nano-materials, as biosensors for pathogen location and as a helpful apparatus against microbes, growths, and infections have furnished a promising contrasting option to manage this subject of concern [8]. The properties of Nanoparticles are ascribed to their high surface zone to volume proportion at nano-measurements.

Silver has been known for its antimicrobial action since the antiquated days. It was utilized for putting away savoring water old circumstances. The definition of silver has changed from mass silver in coins, supplanted by ionic silver to the current colloidal silver. Silver, regardless of whether in ionic or nanoparticle frame, is exceedingly dangerous to microorganisms [15]. It is the most dangerous metal known for its action against microorganisms. Other metals take after silver in regard of antimicrobial movement as shown below:

"Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn"

Besides, silver in nanoparticle frame is more effective than silver particles regarding its antimicrobial activity. Adding to that, it is likewise known to apply bring down poisonous quality to mammalian cells as well [7]

Although antibacterial properties have long been known, the use of silver for serious weaknesses has been mitigated by the disclosure of antibiotics [16]. Although the onset of overwhelming contamination by harmless immunotoxic, toxic, pathogenic microorganisms has taken into account silver and its colloidal forms. At present, clothing, respiratory protection, family water channels, contraceptives, antibacterial showers, pearls, chemicals, nutritional supplements, cell phones, PC consoles and toys for young people are abusing the antimicrobial properties of silver anomalies.

Although antibacterial properties have long been known, the use of silver for serious weaknesses has been mitigated by the disclosure of antibiotics [16]. Although the onset of overwhelming contamination by harmless immunotoxic, toxic, pathogenic microorganisms has taken into account silver and its colloidal forms. At present, clothing, respiratory protection, family water channels, contraceptives, antibacterial showers, pearls, chemicals, nutritional supplements,

cell phones, PC consoles and toys for young people are abusing the antimicrobial properties of silver anomalies.

So far, several clusters of particles included in the mix with the granular lamb with green plant tissue systems aim to natural elements, plants, micro-organisms, etc. Some of those things he does, he describes the subject matter per plant.

Synthesis of Silver Anion Particles (AgNP) Using *Polyalthia longifolia* plates and D-sorbitol as the reducing agents to remove motifs, S. Kaviya et al. It is used to increase the frenzy of nanoparticles represented by [19]. The experiment was performed with two exceptional compression tanks (10⁻³ M and 10⁻⁴ M) of silver nitrate. The effect of the temperature of the AGNPS mixture is examined by mixing at room temperature (25 ° C) and UV spectra at 60 ° C, NP showing a pronounced blue movement growth temperature in two main areas of interest. The FT-IR study showed that the expected biological value still plays a large role in the reduction of Ag + particles and in the development of AgNP. The size and morphology of the nanoparticles was solved by TEM. It was observed that the silver anor particles are in a considerably toxic consciousness against the gram-positive gram-negative microorganism-sensitive life form.

In another paper, the following study reports were found. The combination of rapid biogenic silver nanoparticle liquid green (AgNP) shows the use of the turbo liquid *Chebula* (*T. chebula*) [14]. The formation of silver nanoparticles during stress, which Plasmon Resonance (SPR) at 452 nm using a visible UV spectrophotometer. Gout money in the *T. chebula* financial nanoparticles isolated were taken within 20 minutes, which was considered potentiometrically. The nanoparticles synthesized represented by the UV spectrum, if desired, Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), electron microscopy (TEM) and atomic energy microscopy (AFM). She developed good antimicrobial (25923 *S. aureus* ATCC) bacteria and Gram-positive bacteria, Gram-negative bacteria (*E. coli* ATCC 25922). Mechanism May Be a Strong Decision for Availability in Money Nanoparticles.

The design, synthesis and imaging of naturally mixed nanomaterials have become a very interesting area. It was shown that the extracellular mixture of gold and silver nanoparticles with the fruit of *Emblica officinalis* (Amla, Indian gooseberry) can be a difficult decision to transfer gold and silver nanoparticles with ecological routes [20]. In the treatment of liquid silver sulphates and destructive letters based on chlorine with natural products from *Emblica officinalis*, a rapid reduction of silver and chlorurane particles is released, which causes the development of very stable silver and gold nanoparticles in solution. The analysis of transmission electron microscopy of silver and gold nanoparticles showed that they had sizes from 10 to 20 nm and from 15 to 25 nm separately.

In another experiment, the leaves of five independent plants (pine, persimmon, ginkgo, magnolia and platanus) used, their metallic silver cutupitippicc eksatracellular combination [23] examined. Friday cutupitippicc rabbis Nikitas clear plan agnea (+) Nikitas (0) 3 the continuous aqueous stability of estimated plant leaves. UV spectroscopy was used to determine the amount of silver nanotechnology. silver leaf juice teacher helped change the color of Magnolia faster and nano level. Interactive temperature of 95 ° C using Magnolia glass for 90% sheet in just 11 minutes is sufficient to make any other changes. cutupitippicc silver inductively coupled plasma spectrometry (ICP) dispersive spectroscopy and X-axis criticality (Eds), Monitoring micreasceapy (SEM), electron transmission micreasceapy (TEM), synthesized representation of the particle analyzer. The normal molecular dose is 15 to 500. And for recruitment and temperature, jyusarinreyum ekeayina lisiinreyum concentration levels can be controlled by changing the kanikakalute. In cosmetics, such as mixed structures more quickly and a method of contamination, human contact with medical applications touched by touch in various areas, can be used to provide the speed of silver cutup.

Integration of eco-compliant nanoparticles through different standards is an assessment of some crops for their ability to bind to silver nanoparticles (AgNP) [22]. It has been found in the report that AgNP is used using stalks that consider the origins of sweet potato at 80 degrees Celsius and 25 degrees Celsius temperature. AgNP is under UV-vis, SEM, FTIR, XRD and EDX spectrophotometers. Antimicrobial progress of AgNP is described in gram-positive (*B. subtilis* and *S. aureus*), gram-negative (*E. coli*) and mushrooms (*S. cerevisiae* and *C. albicans*). At room temperature, *S. cerevisiae* and *C. albicans* are better with AgNP than 80 degrees Celsius [24]

A biogenic that combinations of silver nanoparticles green *Foeniculum vulgare* accent of its activities and *Staphylococcus aureus* and *Escherichia coli* have been reported. This study revealed the main silver of nanoparticles by shades of green in the dark after treatment with AgNO₃ (1) and the UV spectrophotometer omistebara control show maximum absorbance at around 427 nm, show a combination of silver nanoparticles [27] must change. and nanoparticle tracking analysis (NTA) of LM-20 for multi-parameter analysis was used, the characteristics of small molecules and silver nanoparticle molecules with a light behind this vulgar *F.* combined ratio. Ss nanoparticles have only different levels of 18-83 nm. silver nanoparticles phyto synthesis changes antibacterial against *Staphylococcus aureus* (ATCC 25923) "and *Escherichia coli* (ATCC 39403) displays. nanoparticles changes bacteria show silver of the same model cause a stain of human life if by mixing the hollow antibiotics are added as a mixture used. bactericidal standard exasperer too microorganisms were everywhere at the nanoparticl has changed the money n against pathogenic bacteria.

For this purpose, medicinal plants are used in the same way. In addition, the green synthesis of silver nanoparticles with paralysis of tobacco leaves was inclined [26]. The synthesized nanoparticles were applied by UV-Vis, TEM, EDAX, FT-IR Amplification Spectroscopy, and a self-adhesive photo-luminescence study. UV-Vision absorption spectroscopy of the planned silver colloidal approach showed a peak of osmosis at 418 nm. From the photoluminescence sampling, the maximum peak of the flare and the greater frequency output were detected at 414 and 576 nm without limits. The TEM analysis showed a typical molecular size of 8 nm, but the SAED approach required the crystalline nature of the synthesized nanoparticles. The EDAX analysis showed that the silver content (54.55%) is nanoparticle elements. *Pseudomonas aeruginosa* and *Escherichia coli* are the most pronounced sensitivity to silver nanoparticles.

The use of silver nanoparticles of citrus concentrations of citrus limon can become an expert in the field of silver nanopartic discrimination [12]. It is a silver nanoparticle used in cotton and silk materials. Headache and antimicrobial effects were found on top With antimicrobial silver nanoparticles which touch the multi-dimensional nature of lemon blades, all silver and lemon are not included in essential oils, and have gone in view of the environmental impact. This report shows that the ionizing cells of the AG + AG0 with the effect of lemon leaves were inspired by war with stable silver nanoparticles. By examining antifungal agents trying to reduce Antifungal therapies, fold the forward fossil oxyporesum and alteria brasicichola of silver nanoparticles. Metal nanoparticles were developed to modify FT-IR, UV-visible spectroscopy, electromechanical and electronic microscopy [28] to test, electromicroscopy and atomic microscopy.

MATERIALS AND METHODS

MATERIALS:

CHEMICALS-

1. Silver Nitrate – Collected from JUIT, Solan
2. Nutrient Agar Powder- Collected from JUIT, Solan
3. Sucrose- Collected from JUIT, Solan
4. Fructose- Collected from JUIT, Solan
5. Ribose- Collected from JUIT, Solan
6. Glucose- Collected from JUIT, Solan

OTHER MATERIALS-

1. De-ionized water – Collected from DI plant

BACTERIAL STRAINS-

1. *Escherichia coli* – Collected from JUIT, Solan

OTHER APPARATUS-

1. Weighing Balance
2. Micropipette
3. Petri dish of 11 cm diameter
4. Centrifuge machine
5. Magnetic Stirrer
6. Autoclave
7. Culture tubes
8. Test tubes
9. Inoculating needle
10. Refrigerator

CHAPTER 3

3.1 Experimental Approach

1. PREPARATION OF SILVER NANOPARTICLES

The synthesis procedure comprises of four simple steps:

1. Preparation of sugar solutions i.e. sucrose, glucose, ribose, fructose
2. Preparation of silver nitrate solutions of different concentrations
3. Addition of the sugar solution to the silver nitrate solutions
4. Incubation at room temperature to allow nanoparticles formation

Protocol:

- Stock solution of AgNO₃ was prepared
- 25ml of AgNO₃ was taken from the stock and transferred to conical flask
- 0.2 % in 50 ml freshly prepared sugar solution was also prepared
- Metal solution and sugar solution was mixed together
- The mixed solution was then kept in direct sunlight for 10 min
- After 10 min colour of the solution was changed to yellow, which is the first indicator for the formation of nanoparticles.
- The solution was then observed after 24 hours

3.1.2 Preparation of sugar solution:

A large number of chemicals has already been used to synthesize metal nanoparticles from metal salts. But the preparation methods of those chemicals were complicated. Here we had used a very easy and effective technique.

a.) Relatively sucrose, fructose, ribose, glucose was taken from the laboratory.

b.) 50 ml of 0.2% i.e. 0.1 g was taken in 250 ml conical flask.

The solution was preserved inside a refrigerator for future use

3.1.3 Preparation of Silver Nitrate solution

Analytical grade silver nitrate (AgNO_3), a costly chemical, was taken from laboratory. A 1 mM stock solution of AgNO_3 in chloride-free distilled water was prepared according to the following calculations.

The molecular weight of AgNO_3 :

[Ag-107.87, N-14, O-16]

$= 107.87 + 14 + (16 \times 3) = 169.87$ Therefore, Molar

mass of $\text{AgNO}_3 = 169.87$ g

Therefore, 25 ml of 1 mM solution will contain 0.00424 g of AgNO_3 :

Calculations:

<u>Density</u>	<u>Volume</u>	<u>Required amount of AgNO_3</u>
1000 mM	1000 ml	169.87 g
1 M	25 ml	$(\text{wt.} \times 169.87) / (1000 \times 25) = 4.24$ g
1 mM	25 ml	$4.24 / 1000 = 0.00424$ g

Weighed amount of AgNO_3 was carefully transferred in a 250-ml conical flask and de-ionized water was added drop-wise while swirling to dissolve the salt up to the mark. The solution was diluted as required and all the solutions were kept away from light (the containers were wrapped with brown papers) and kept in dark.

3.1.4 Preparation of silver nanoparticles by sugar solution to AgNO₃ solutions

Before addition of fruit extract to the AgNO₃ solution, the volume of 1 mM AgNO₃ solution required to attain a specific concentration (5mM, 10mM, 20mM and 25mM) of the salt was calculated as follows

Volume of sugar solution	Volume of AgNO ₃	Final volume of solution
50 ml	25 ml	75 ml
50 ml	25 ml	75 ml
50 ml	25 ml	75 ml
50 ml	25ml	75 ml

Table 3.1 shows a set of four conical flasks were taken and marked. In four conical flasks requisite volumes of AgNO₃ solution and sugar solution were added one after another as shown in the table above; The contents were mixed thoroughly and left at room temperature in dark

CHAPTER 4

4 RESULTS:



Fig. 4.1 Following image shows initial color of the mixtures in the four conical flasks

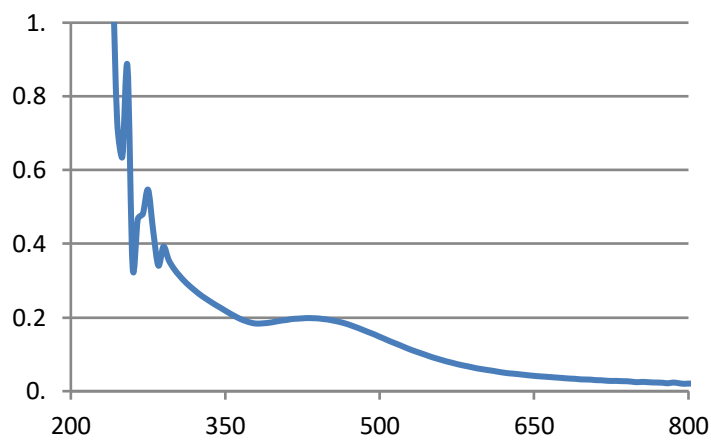


Fig 4.2 Colors of samples after mixing and keeping the solutions in sunlight

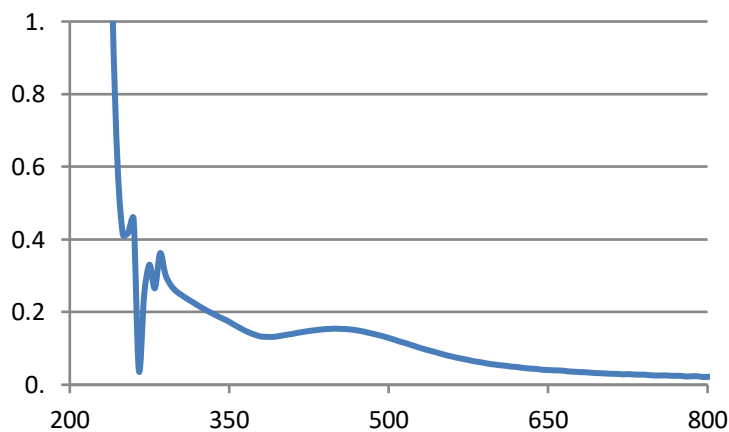
4.2 CHARACTERIZATION OF SILVER NANOPARTICLES

4.2.1 UV-Vis Spectroscopy

After addition of sugar answer for the fluid arrangement of AgNO_3 of various focuses, the blend demonstrated a steady change in shading at room temperature with time from yellowish to darker and the shading heightened following 48 hours. The decrease of silver particle to silver nanoparticle was reflected in unearthy information got by utilizing an UV-Vis spectrophotometer. It demonstrates an absorbance top around 450 nm for each of the four examples which is particular for silver nanoparticles



FFig 4.2.1 The absorbance spectrum of glucose silver nanoparticles showing maximum absorbance near 450nm



FigFig 4.2.2 The absorbance spectrum of sucrose silver nanoparticles showing maximum absorbance near 450nm

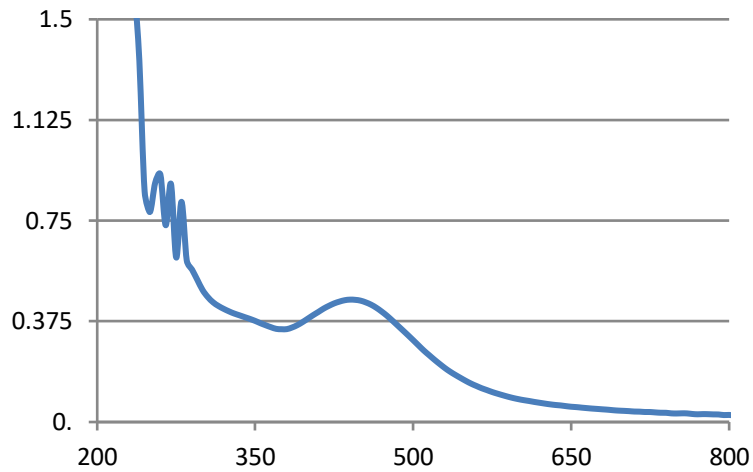


Fig 4.2.3 The absorbance spectrum of ribose silver nanoparticles showing maximum absorbance near 450nm

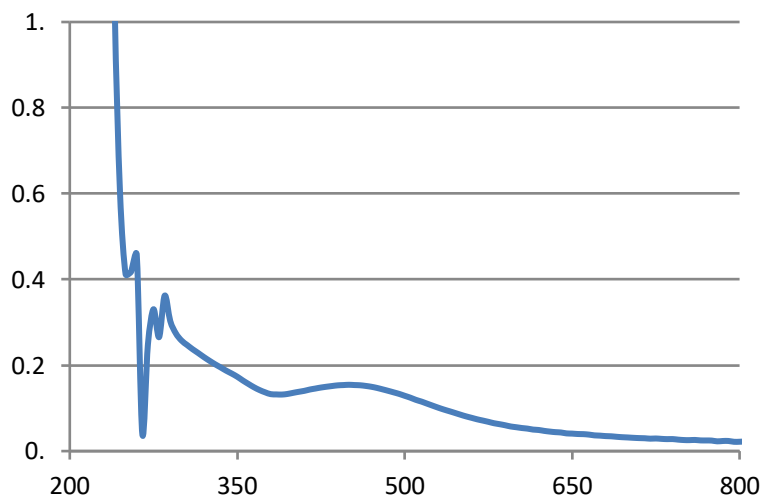


Fig 4.2.4 The absorbance spectrum of fructose silver nanoparticles showing maximum absorbance near 450nm

4.3 SEM Results:

Scanning Electron Microscopy is improved the situation uncovering the surface morphology of particles. Here, the dab for the SEM examination was set up by setting a drop of the silver nano-molecule suspension on the carbon tape connected to the head of tube shaped dab and it was dried

inside a vacuum dryer for two or three hours. The particles on the highest point of the dot were filtered by Scanning Electron Microscope and the accompanying picture was acquired

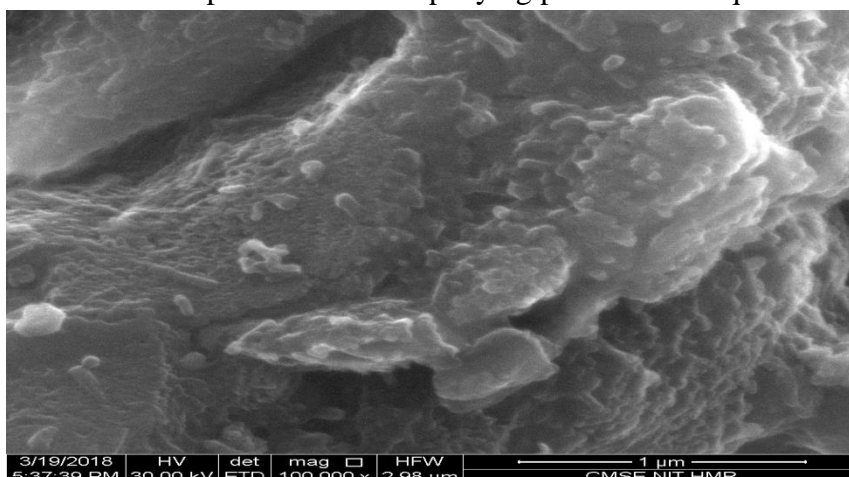


Fig 4.3.1 SEM image showing surface morphology of fructose silver nanoparticles

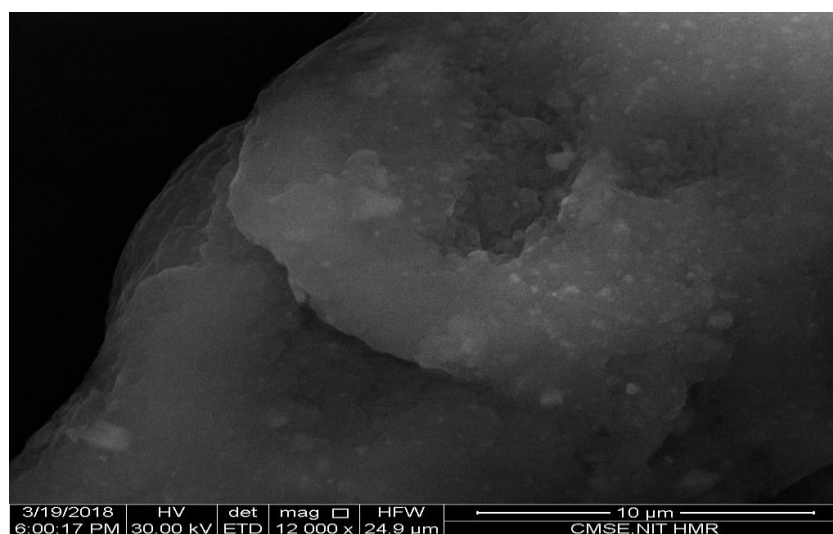


Fig 4.3.2 SEM image showing surface morphology of sucrose silver nanoparticles

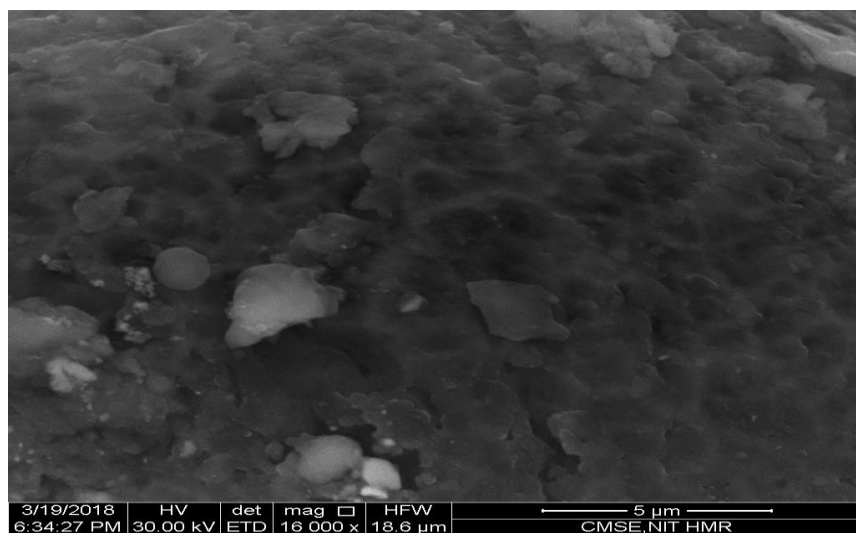


Fig 4.3.3 SEM image showing surface morphology of glucose silver nanoparticles

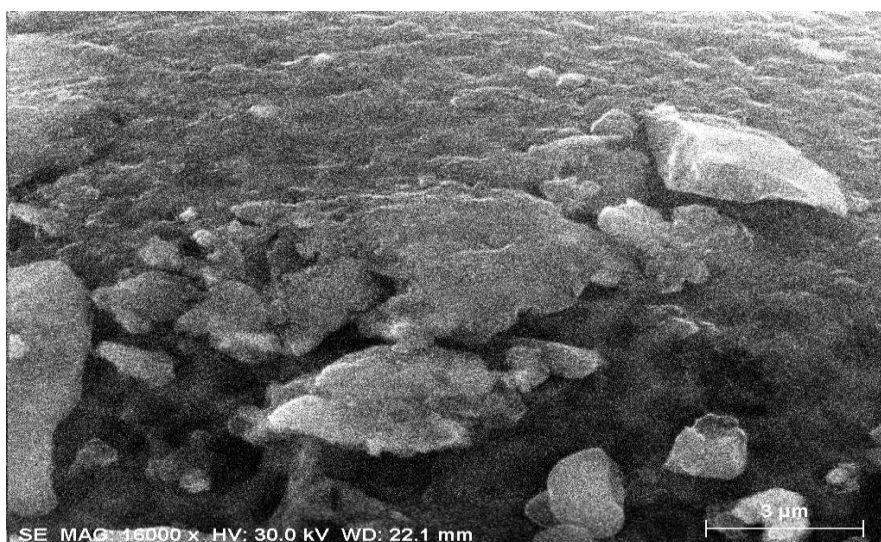
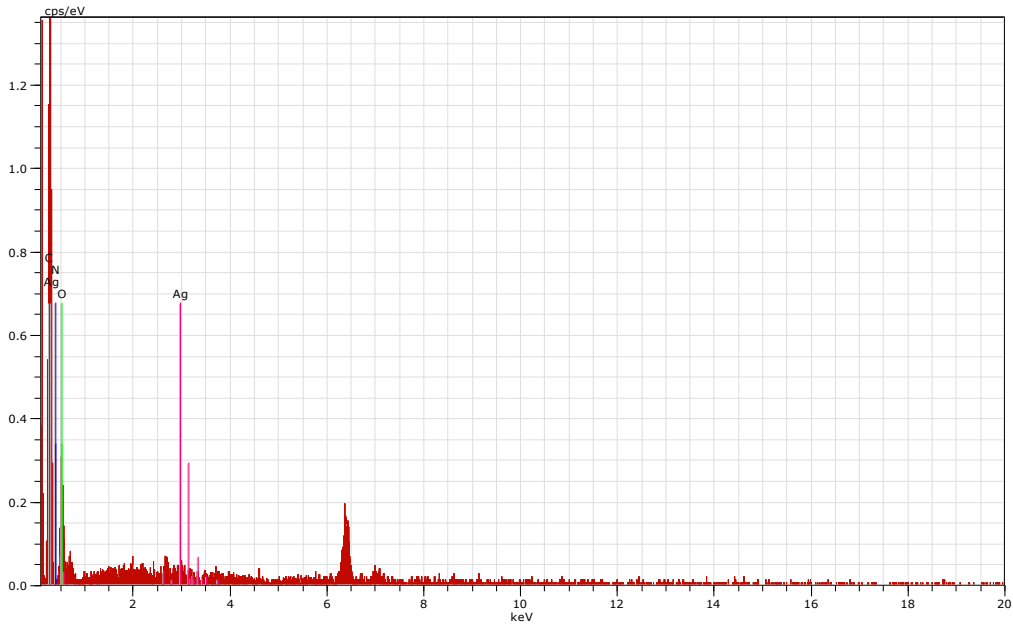


Fig 4.3.4 SEM image showing surface morphology of ribose silver nanoparticles

4.4 Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy or EDX is a system that is primarily used to recognize the nearness of various components in an example. It is important to confirm the nearness of wanted component in an example. In the present investigation, this strategy was utilized to check the

nearness of Ag and the bend demonstrated a little pinnacle of the component alongside those of C



and O"

Fig 4.4.1 EDX curve of fructose Ag-nanoparticles showing the presence of Ag and other elements (C and O).

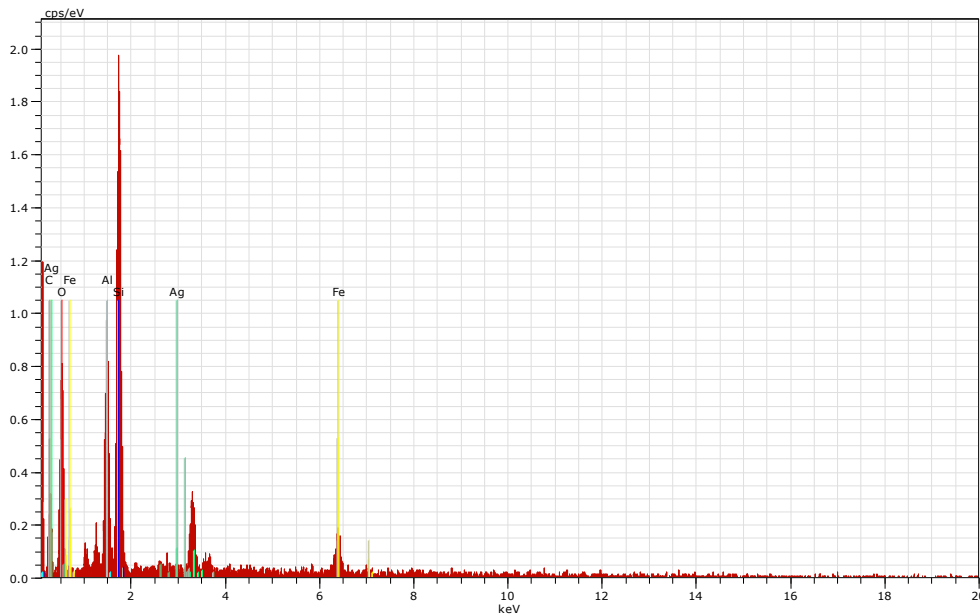


Fig 4.4.2 EDX curve of sucrose Ag-nanoparticles showing the presence of Ag and other elements (C and O).

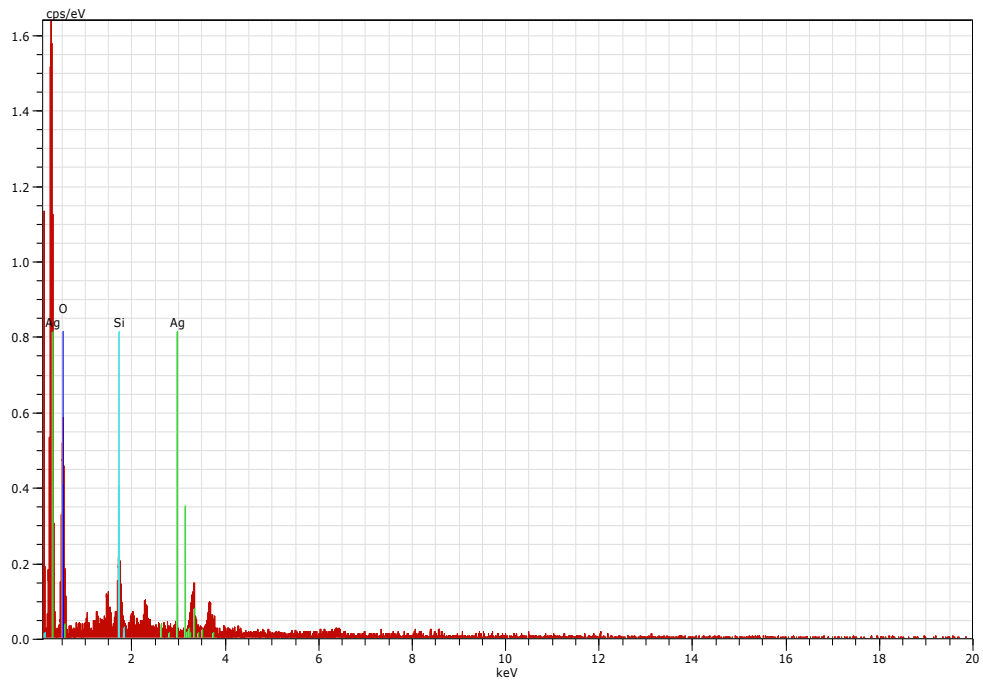


Fig 4.4.3 EDX curve of glucose Ag-nanoparticles showing the presence of Ag and other elements (C and O).

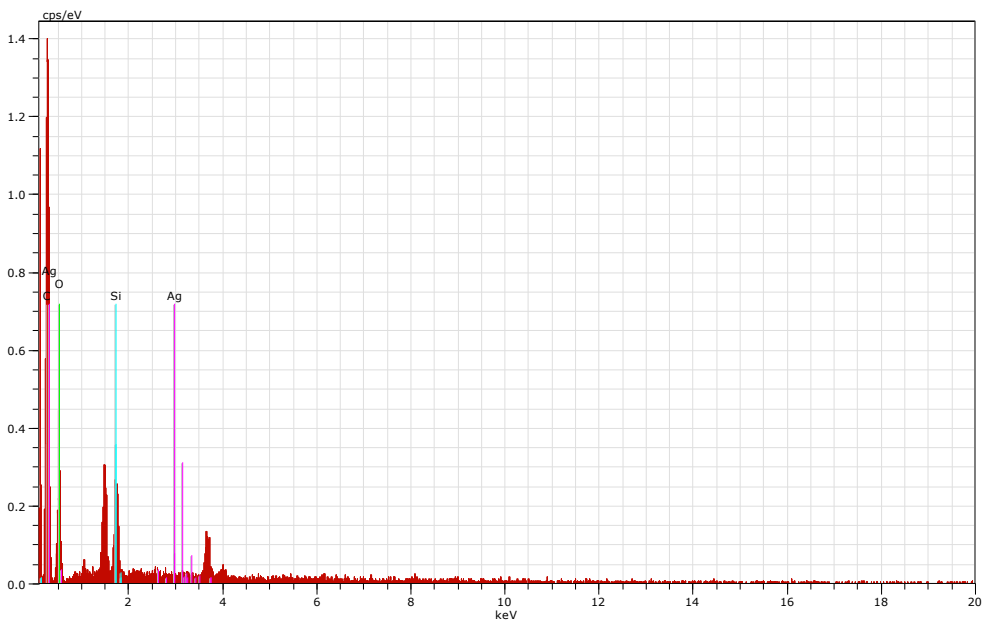


Fig 4.4.4 EDX curve of ribose Ag-nanoparticles showing the presence of Ag and other elements (C and O).

4.5 Observation of zone of inhibition

The following images show zone of inhibition as observed during the experiment

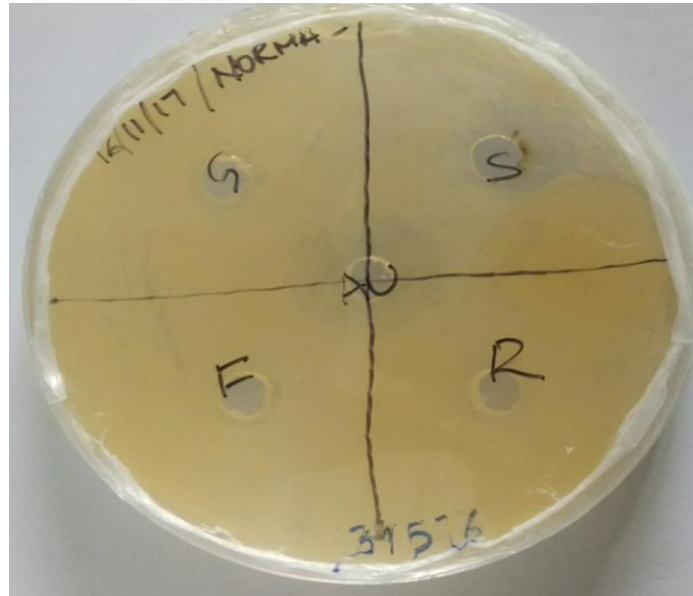


Fig 4.5.1 The disk D1 shows Inhibition Zone for sugar solution where negative control shows no result

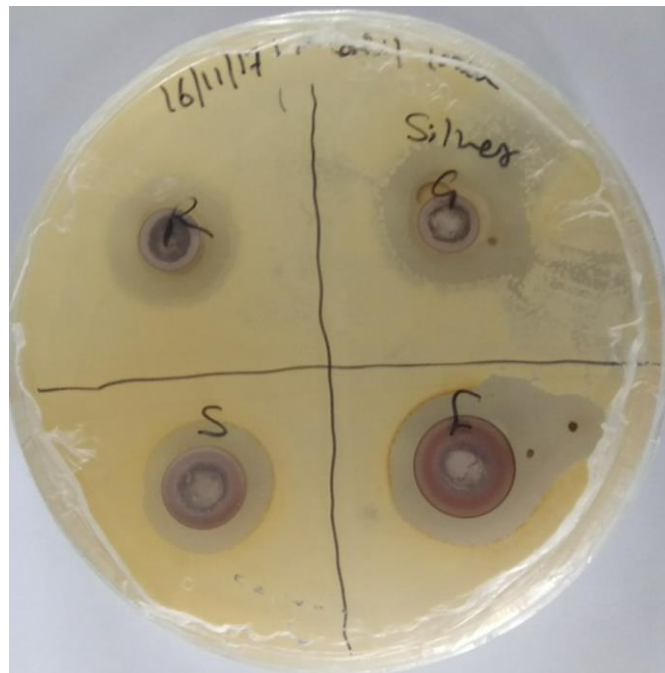


Fig 4.5.2 The disk D2 shows Inhibition Zone for AgNP's where negative control shows no result

The distinct Zone of Inhibition was seen around the glass wherein the suspension of AgNPs was connected as appeared previously. The blends of AgNO₃ and sugar arrangement with 1 mM and 2 mM focuses additionally kept the bacterial development. The sterile sugar arrangement demonstrated no outcome. The same microbiological test was performed against normal microscopic organisms *E. coli* yet the organically integrated silver nanoparticles did not demonstrate any positive outcome.

At the end of this antimicrobial screening test, it is affirmed that the organically combined Silver Nanoparticles (SNP) have successful antibacterial property. In this manner, uses of SNPs can cover an extensive space of restorative, cowhide and sustenance innovations.

4.6 Study of catalytic action of prepared AgNP's against Methyene Blue

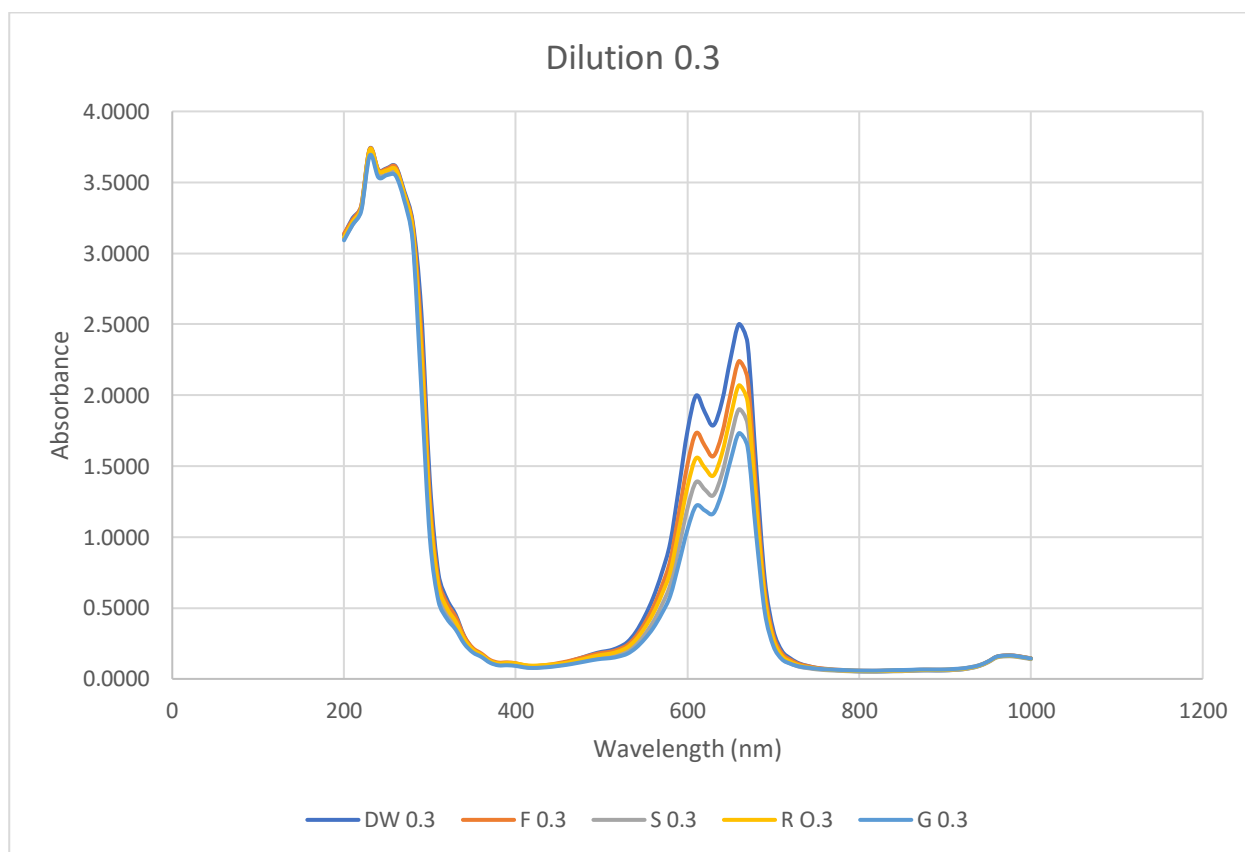


Fig 4.6.1 shows the catalytic breaking point of AgNP's was asked about utilizing the diminishment development of MB by NaBH₄ as a model reaction. MB has blue shading in oxidized state and loses it's shading upon decrease encompassing depleting leucomethylene blue. The UV-Vis spectra of lessening of MB by NaBH₄ without impetus 'AgNPs' is appeared in above Fig. the UV-Vis spectra of MB diminishment by NaBH₄ inside observing coordinated force 'AgNPs' i.e. 0.3 ml

CONCLUSION

The target set for this examination is to integrate AgNP's in a clear and simpler path, not at all like that of concoction combination. We utilized particular sugars as a decreasing and topping specialist. This technique encourages us to combine silver nanoparticles in significantly more simpler way. I effectively depicted the normally combined Ag-nanoparticles. These Ag nanoparticles showed antibacterial activity against E. coli

A couple of usages of these Green Silver Nanoparticles are:

1. Because of its antibacterial activity against E.coli, these nanoparticles can help in therapeutic use.
2. Due to its conductive properties, we can complete these AgNP's in front-line compact contraptions.
3. Nanoparticles can demonstrate exceptionally valuable for the general public and in future, it can help in the field of therapeutic science since it can help in treating different sicknesses which till now has no cure.

REFERENCES

- [1] OV Salata “Applications of nanoparticles in biology and medicine”, *Journal of Nanobiotechnology*, vol. 2, pp. 2-3, 2004
- [2] Colin Pettegrew, Zheng Dong, M. Zubayed Muhi, Scott Pease, M. Abdul Mottaleb, and M. Rafiq Islam “Silver Nanoparticle Synthesis Using Monosaccharides and Their Growth Inhibitory Activity against Gram-Negative and Positive Bacteria” *Journal of ISRN Nanotechnology* Vol.2014 (2014), 8
- [3] Qifeng Chen, Guhong Liu, Guangxue Chen, Ting Mi, Jinglei Tai “Green Synthesis of Silver Nanoparticles with Glucose for Conductivity Enhancement of Conductive Ink” *Journal of BioResources*, Vol 12, pp. (2017)
- [4] Emanuela Filippo, Antonio Serra, Alessandro Buccolieri, Daniela Manno “Green synthesis of silver nanoparticles with sucrose and maltose: Morphological and structural characterization”, *Journal of Non-Crystalline Solids*, Volume 356, pp. 344-350, 2010
- [5] Germán Ayala, Luci Cristiane de Oliveira Vercik, Thiago Antônio Villa Menezes and Andrés Vercik “A Simple and Green Method for Synthesis of Ag and Au Nanoparticles using Biopolymers and Sugars as Reducing Agent”, *Journal of Materials Research Society*, vol. 1386, pp., 2012
- [6] Poovathinthodiyil Raveendran, Jie Fu and Scott L. Wallen “A simple and “green” method for the synthesis of Au, Ag, and Au–Ag alloy nanoparticles”, *Journal of Green Chemistry*, vol. 8, pp. 34-38, 2006
- [7] Sukumaran Prabhu, Eldho K Poulse “Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects”, *Journal of International Nano Letters*, vol. 6, pp. 22-26, 2012
- [8] Irshad Ahmad Wani “Biomedical Applications of Gold Nanoparticles: Recent Advances and Future Prospects”, *Journal of Biomedical Engineering*, vol. 37, pp. 1-22, 2018
- [9] Christian Engelbrekt, Karsten H. Sørensen, Jingdong Zhang, Anna C. Welinder, Palle S. Jensen, Jens Ulstrup “Green synthesis of gold nanoparticles with starch–glucose and application in bioelectrochemistry” *Journal of Material Biochemistry*, vol. 19, pp. 7839-7847, 2009
- [10] Kavita K. Katti, Vijaya Kattumuri, Sharanya Bhaskaran, Kattesh V. Katti, Raghuraman Kannan “Facile and General Method for Synthesis of Sugar Coated Gold Nanoparticles”, *International J journal Green Nanotechnol Biomed*, vol. 1, pp. 53–59, 2009
- [11] Zaheer Khan, Shaeel Ahmed Al-Thabaitia, Ommer Bashir “Natural sugar surfactant capped gold nano-disks: Aggregation, green synthesis and morphology”, *Journal of Dyes and Pigments*, vol. 124, pp. 210-221, 2016

- [12] Majid Darroudi, Mansor Bin Ahmad, Abdul Halim Abdullah, Nor Azowa Ibrahim “Green synthesis and characterization of gelatin-based and sugar-reduced silver nanoparticles”, *International Journal of Nanomedicine*, vol. 6, pp. 569–574, 2011
- [13] Chengzhou Zhu, Shaojun Guo, Youxing Fang, Shaojun Dong “Reducing Sugar: New Functional Molecules for the Green Synthesis of Graphene Nanosheets”, *Journal of ACS Nano*, vol. 4, pp. 2429–2437, 2010
- [14] Ragavendran Abbai, Ramya Mathiyalagan, Josua Markus, Yeon-Ju Kim,² Chao Wang, Priyanka Singh, Sungeun Ahn, Mohamed El-Agamy Farh, Deok Chun Yang “Green synthesis of multifunctional silver and gold nanoparticles from the oriental herbal adaptogen: Siberian ginseng”, *International Journal of Nano medicine*” vol. 11, pp. 3131-3143, 2016