SYNTHESIS OF NANOPARTICLES USING *LANTANA CAMARA* AND STUDY OF THEIR PHOTOCATALYTIC ACTIVITY

By

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A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Chemistry

Department of Chemistry

CHITTAGONG UNIVERSITY OF ENGINEERING AND TECHNOLOGY

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Declaration

I hereby declare that the work contained in this Thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the Thesis contains no material previously published or written by another person except where due reference is cited. Furthermore, the Thesis complies with PLAGIARISM and ACADEMIC INTEGRITY regulation of CUET.

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Dedication

To my beloved parents and teachers, infinitely supportive

Conference

Conference

- Syed Julkar Nine* and M.K. MD. Ziaul Hyder, "Green Synthesis Silver Nanoparticles with *Lantana camara* and Study of Photocatalytic Activity. 1st National Conference on Advances in Science and Technology (1st NCAST), Bangladesh University of Engineering & Technology, 07-08 December 2023.

Approval/Declaration by the Supervisor

This is to certify that Syed Julkar Nine has carried out this research work under my supervision, and he has fulfilled the relevant Academic Ordinance of the Chittagong University of Engineering and Technology, so that he is qualified to submit the following Thesis in the application for the degree of Master of Science in Chemistry. Furthermore, the Thesis complies with the PLAGIARISM and ACADEMIC INTEGRITY regulation of CUET.

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CERTIFICATION

The thesis titled "SYNTHESIS OF NANOPARTICLES USING *LANTANA CAMERA* AND STUDY OF THEIR PHOTOCATALYTIC ACTIVITY" submitted by **Syed Julkar Nine**, Student ID: **20MSCHEM002F** of Session: 2020-2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of **Master of Science in Chemistry** on 12 December, 2023.

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Abstract

Nanoscience and nanotechnology have been conquering themselves in the field of research through the future perspective for the modern world. Nanoparticles are one of the fabulous aspects of nanotechnology and nanoscience having at least one dimension in the nanoscale range between 1-100 nm with a very high surface to volume ratio. Considering metallic nanoparticles, silver nanoparticles (AgNPs) are gaining interest day by day because of their broad area of applications in different fields such as pharmaceutical, medicinal, catalysis, and wastewater treatment. Approaching environment-friendly procedures, plant-mediated synthesis is one of the green synthesis methods with less toxicity, easy production, cost-effectiveness, and free from hazardous chemicals. This present study aimed towards the synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Lantana camara* with silver nitrate ($AgNO_3$) as the precursor. Synthesis of AgNPs was confirmed by a UV-Visible spectrophotometer by observing the Surface Plasmon Resonance (SPR) band peak at 458 nm followed by its color changes from light yellow to dark brown of the reaction media. Fourier Transformed Infrared (FTIR) showed the structure and respective bands of the synthesized nanoparticles and the stretch of bonds. Surface morphology and dispersity were characterized using a Field Emission-Scanning Electron Microscope (FE-SEM) and the particles were observed spherical without agglomeration and the particle size was found approximately in the range between 15 to 35 nm. The purity and crystalline nature of AgNPs were revealed by Energy Dispersive Spectroscopy (EDS) and X-ray Diffraction (XRD) analysis, respectively. Photocatalytic activity of AgNPs was observed against Methylene blue (MB) dye under solar light irradiation. Green synthesized silver nanoparticles effectively degraded the dye by nearly 95% at 5 h of exposure time. The degradation of dye at various concentrations was fitted to Langmuir and Freundlich isotherm models and the data showed best fitting toward Freundlich isotherm model. The degradation of dye with the time was fitted to pseudo-first-order and pseudo-second-order kinetic models where the data best fit the pseudo-first-order kinetic model. Synthesized AgNPs showed moderate anti-microbial activity against four bacterial strains (gram-positive and gram-negative) while they showed very good activity against two anti-fungal strains.

বিমূর্ত

ন্যানোসায়েন্স এবং ন্যানোটেকনোলজি আধুনিক বিশ্বে ভবিষ্যত দৃষ্টিভঙ্গির মাধ্যমে গবেষণা ক্ষেত্রে একটি সুদৃঢ় অবস্থান গঠিত করছে. ন্যানো পার্টিকেল হল ন্যানোপ্রযুক্তি এবং ন্যানোসায়েন্সের একটি চমত্কার দিক, যার আকার অন্তত একমাত্রিক দিক থেকে ন্যানোস্কেল পরিসরে 1-100 এনএম এর মধ্যে এবং খুব উচ্চ পৃষ্ঠ আয়তনের অনুপাত রয়েছে। ফার্মাসিউটিক্যাল, মেডিসিনাল, ক্যাটালাইসিস এবং বর্জ্যপানি নিষ্কাশনের মতো বিভিন্ন ক্ষেত্রে বিস্তার প্রয়োগের কারণে সিলভার ন্যানো পার্টিকেলস (AgNPs) নিয়ে দিন দিন গবেষণার আগ্রহ বাড়ছে। উদ্ভিদের সাহায্যে সংশ্লেষণ অন্যতম একটি সবুজ সংশ্লেষণ পদ্ধতি পরিবেশ বান্ধব পদ্ধতিগুলির মধ্যে যা কম বিষাক্ততা যুক্ত, সহজ উৎপাদন, সহজলোভ্য এবং যেকোনো ধরণের বিপজ্জনক রাসায়নিক থেকে মুক্ত। এই অধ্যয়নের মূল লক্ষ্য হলো, *Lantana camara* গাছের পাতার জলীয় নির্যাস ব্যবহার করে সিলভার নাইট্রেট ($AgNO_3$) এর সাহয্যে সিলভার ন্যানো পার্টিকেল (AgNPs) এর সংশ্লেষণ। AgNPs এর সংশ্লেষণ, বিক্রিয়া মাধ্যমের রঙ হালকা হলুদ থেকে গাঢ় বাদামীতে পরিবর্তন এবং একটি অতি বেগুনি-দৃশ্যমান স্পেকট্রোফোটোমিটার দ্বারা 458 nm এ সারফেস প্লাজমন রেজোন্যান্স (SPR) ব্যান্ড পিক পর্যবেক্ষণ করে নিশ্চিত করা হয়েছিল। FT-IR দ্বারা সংশ্লেষিত সিলভার ন্যানোপার্টিকেল এ উপস্থিত বন্ধনের প্রসারণ এবং গঠন শনাক্ত করা হয়। ন্যানোপার্টিকেলের আকার এবং তলের ধরণ ফিল্ড এমিশন-স্ক্যানিং ইলেক্ট্রন মাইক্রোস্কোপ (FE-SEM) ব্যবহার করে নির্ণয় করা হয়েছিল যেখানে কণাগুলিকে গোলাকার এবং পরস্পরের সাথে অগুচ্ছ অবস্থায় পর্যবেক্ষণ করা হয়। কণার আকার প্রায় 15 থেকে 35 এনএম এর মধ্যে পাওয়া গেছে। AgNPs এর বিশুদ্ধতা এবং স্ফটিকের প্রকৃতি যথাক্রমে এনার্জি ডিসপারসিভ স্পেকট্রোস্কোপি (EDS) এবং এক্স-রে ডিফ্র্যাকশন (XRD) বিশ্লেষণের মাধ্যমে পর্যবেক্ষণ করা হয়েছিল। AgNPs এর ফটোক্যাটালিটিক সক্রিয়তা methylene blue (MB) রঞ্জকের উপর সৌর আলো বিকিরণের অধীনে পরিলক্ষিত করা হয়েছিল। সবুজ পদ্দতিতে সংশ্লেষিত সিলভার ন্যানো পার্টিকেলগুলির সক্রিয়তা 5 ঘন্টা পর্যন্ত সৌর আলোর অধীনে পরিলক্ষিত করা হয় এবং তা কার্যকরভাবে রঞ্জককে প্রায় 95% অবনমিত করে। ল্যাংমুর এবং ফ্রুন্ডলিচ আইসোথার্ম মডেলগুলির সাথে বিভিন্ন ঘনত্বে রঞ্জক দ্রবণের অবনমন তুলনা করা হয়েছিল এবং তথ্যগুলি ফ্রুন্ডলিচ আইসোথার্ম মডেলের সাথে সর্বোত্তম মানানসই দেখায়। সময়ের সাথে রঞ্জকের অবনমন ছদ্ম-প্রথম-ক্রম এবং ছদ্ম-দ্বিতীয়-ক্রম গতিবিদ্যা মডেলগুলির সাথে তুলনা করা হয়েছিল। যেখানে তথ্যগুলি ছদ্ম-প্রথম-ক্রমের সাথে সর্বোত্তম মানানসই দেখায়। সংশ্লেষিত AgNPs এর এন্টি-মাইক্রোবিয়াল সক্রিয়তা চারটি ব্যাকটেরিয়াল স্ট্রেনের (গ্রাম-পজিটিভ এবং গ্রাম-নেগেটিভ) বিরুদ্ধে পর্যবেক্ষণ করা হয় যা পরিমিত সক্রিয়তা দেখায় এবং দুটি অ্যান্টি-ফাঙ্গাল স্ট্রেনের বিরুদ্ধে খুব ভাল সক্রিয়তা দেখায়।

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Nomenclature

Symbols

Symbols	Elaborations
e.g.	for example
etc.	Etcetera
et al	And Others
Fig.	Figure
E. coli	*Escherichia coli*
A. niger	*Aspergillus niger*
S. aureus	*Staphylococcus aureus*
B. megnaterium	*Bacillus megnaterium*
S. typhi	*Salmonella typhi*
T. harzianum	*Trichoderma harzianum*
nm	nanometer

Acronyms and Abbreviations

NP	Nanoparticles
AgNPS	Silver nanoparticles
MB	Methylene Blue
UV	Ultra Violet
FT-IR	Fourier Transform-Infrared spectroscopy
FE-SEM	Field Emission-Scanning Electron Microscopy
EDX	Energy Dispersive x-ray
XRD	State of the Environment
PE	Plant Extract
DMSO	Dimethyl Sulfoxide
MHA	Mueller Hinton Agar
PDA	Potato Dextrose Agar

Chapter 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Nanotechnology completes the function of nanostructures by creating atoms, molecules, and supramolecules within the nanostructures. The technology pledges scientific progress in the field of modern research of fabrication, manipulating, and individualizing nanoparticles. (Rotello, 2004). A nanoparticle or discerning particle is usually described as a particle with a diameter of between 1 and 100 nm. The term is sometimes used for large particles up to 500 nm or for fiber and tubes less than 100 nm in only two dimensions. (Moodley et al., 2018). These particles have special properties in terms of their very tiny size and a very large and high surface to volume ratio which are completely different from their bulk counterparts.

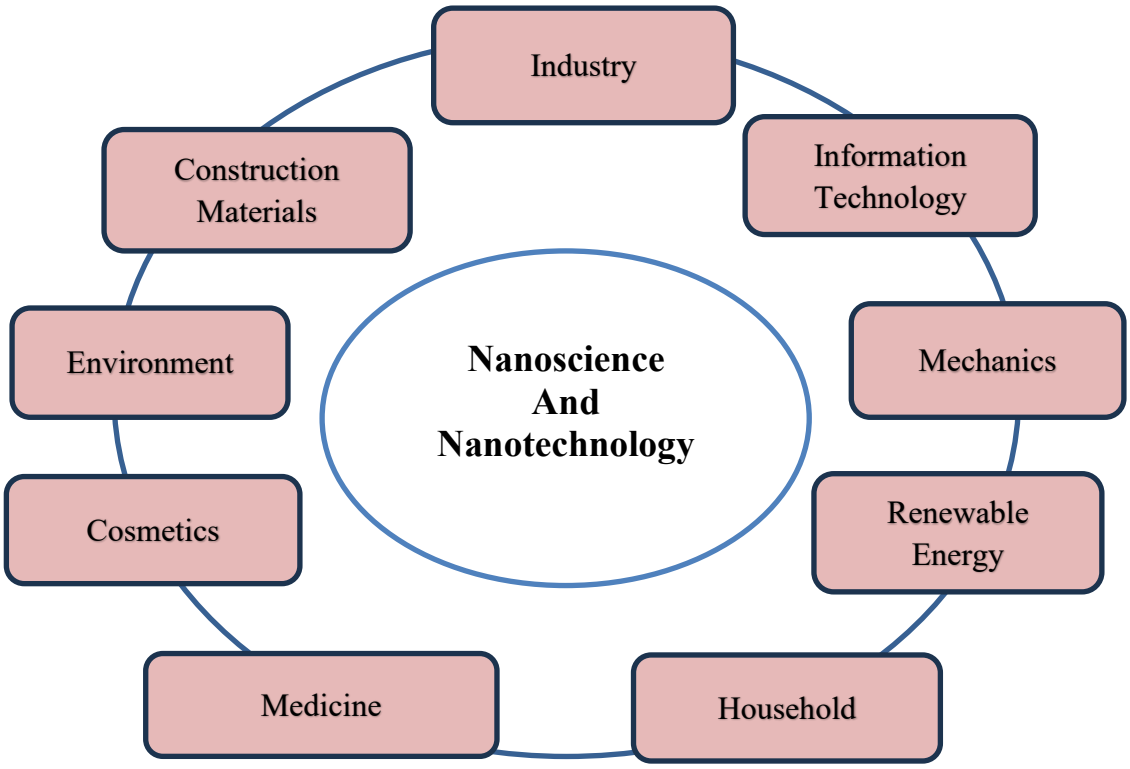


Figure 1.1 Fields of nanoscience and nanotechnology

The area of nanotechnology has seen fascinating progress in various fields such as integration of nanoscale matter and exploiting their physicochemical and optoelectronic forms. In the field of biomedical, electronics, food and health care, drug-gene delivery, the importance of nanotechnology is increasing day by day. (Pirtarighat et al., 2019)

Nanoparticles are found in various forms such as metal, metal oxide, silicates, non-oxide ceramics, carbon, polymers, biomolecules, and composites materials. The morphology of these materials is different from one another. Nanoparticle exists in spheres, disks, cylindrical, platelet, and tube shaped. (Nagarajan, 2008)

The properties of nanoparticles are varied with their morphology. Numerous metals are found in nature but all of them silver, gold, platinum, and palladium synthesized in nanostructure formed. Staggering physicochemical, electrical, optical, and magnetic peculiarities are shown by noble metal nanoparticles. Due to their extraordinary properties, they are extensively used in various fields. Among noble metal nanoparticles, silver is synthesized widely because of its identical features. The salient characteristics have made them proper to use in pharmaceutics, agriculture, water detoxification and filtration, air filtration, and dye degradation in the textile industry, catalysis, and chemical industries. (Sathishkumar et al., 2009)

Nanoparticles have some emerging properties such as large surface area, faster rates to equilibrium, less resistance to diffusion, and high adsorption. Because of these properties nanoparticles have gained great importance in the last few decades. (Marimuthu et al., 2020). Researchers explored the application of silver nanoparticles as a dye-removal agent. Having some supercilious physical, chemical, and biological properties silver nanoparticle is very versatile for implementation in various industries. Silver nanoparticles possess also antibacterial and anti-fungal besides these mentioned characteristics and facilitate their application in wastewater treatment. (Marimuthu et al., 2020)

Nanoparticles are being engineered to enhance their strength, catalytic behavior, morphology, and surface properties. Implantation of nanoparticles as a way of therapeutic or diagnostic cures in the treatment of some diseases related to the brain which are incurable, making this possible because of some features of nanoparticles including developing non-disruptive delivery systems with the size of nano, magnetic, paramagnetic, photothermal, photoluminescence, dispersing and electrical characteristics.

There are some conventional synthesis processes like physical and chemical methods but these methods have some major drawbacks. Using the physical method to synthesize nanoparticles, a large number of particles can be produced e.g., controlled yield and product can be gained in powder form with fine particles. But it has some disadvantages such as very time consuming and large space needed. (Natsuki et al., 2015)

On the other hand, the chemical synthesis method involves toxic and hazardous materials, which is not an environment-friendly method. And also prohibited for medical uses as an application. Because nanoparticles synthesized via this route bind chemicals on their surface. (Pirtarighat et al., 2019).

AgNPs can be synthesized through chemical methods with high yield and narrow size distribution of particles. (Natsuki et al., 2015).

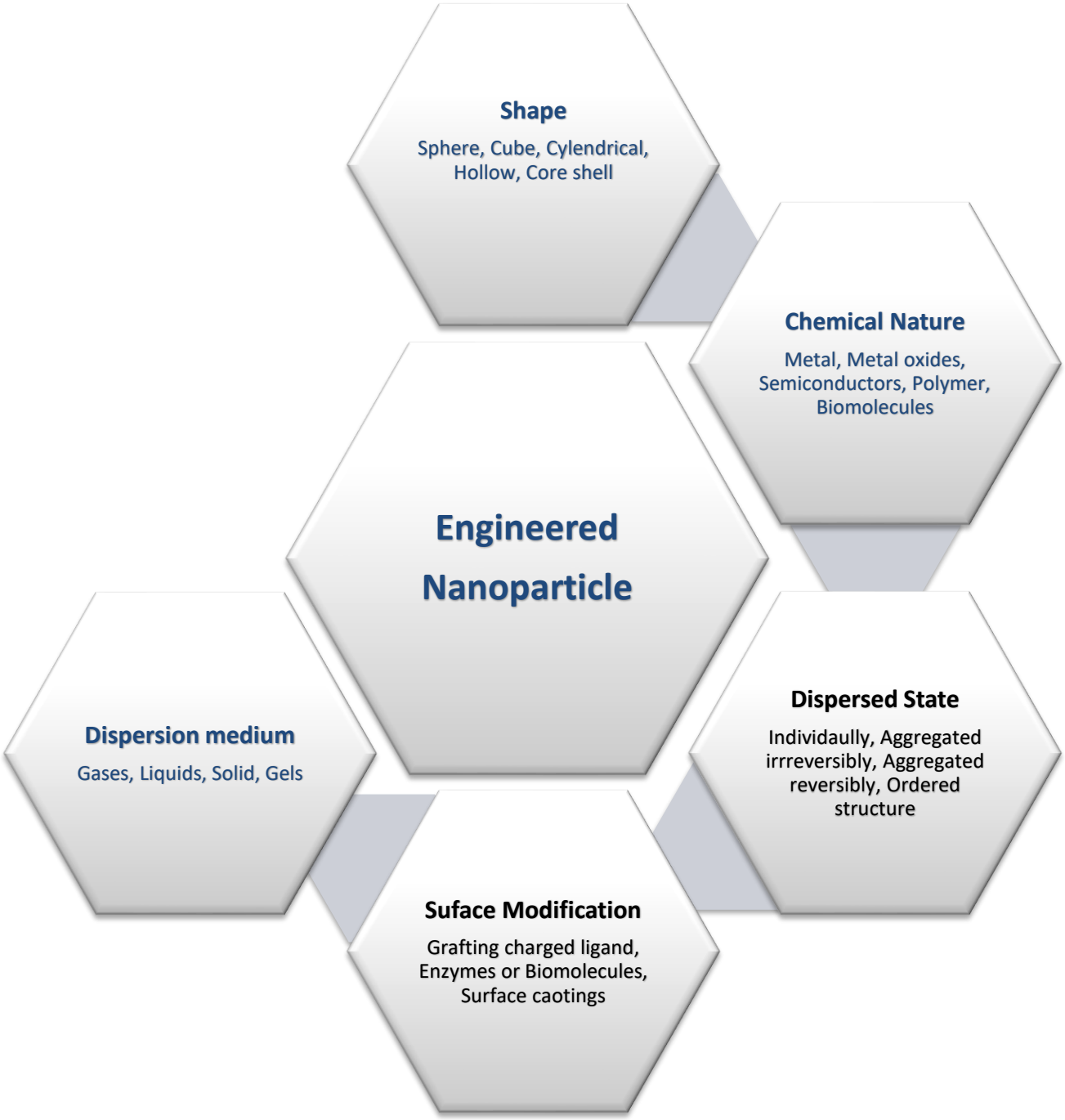


Figure 1.2 Different properties of engineered nanoparticles with their diversity

Other various methods which include toxic organic solvents and strong reducing agents for the synthesis of resultant hazardous and toxic wastes, becoming a great harm for the environment. (Brust et al., 1994; H. et al., 2007; Wu & Lai, 2004)

Table 1.1. Various synthesis methods of AgNPs

Synthesis process	**Limitations**	**References**
Physical method	Time consuming Expensive Large space	(Pirtarighat et al., 2019)
Chemical method	Hazardous and toxic chemicals involve High energy Toxic byproducts	(Khalil et al., 2016)
Photochemical method	High cost High energy	(Natsuki et al., 2015)
Sol-gel method	Time consuming Uses of toxic organic solvent Remaining of hydroxyl/carbon group after the reaction Expensive High temperature	(Park & Ruoff, 2009)
Hydrothermal process	Need high temperature Reaction time is long	(Liu et al., 2014)
Electrothermal process	Multistep synthesis	(Govindhan et al., 2015)
Ex-situ reduction process	Multistep synthesis Time consuming	(Yin et al., 2015; Zhuo et al., 2013)

An eco-friendly synthesis method is necessary to overcome these conventional methods. (Gour & Jain, 2019; Patra & Baek, 2015; Rajasekhar & Kanchi, 2018; Salem & Fouda, 2021). Without any of the toxic and hazardous chemical or any kind of exterior agents an environmentally friendly processes being developed by the researcher recently as safe way of approach.

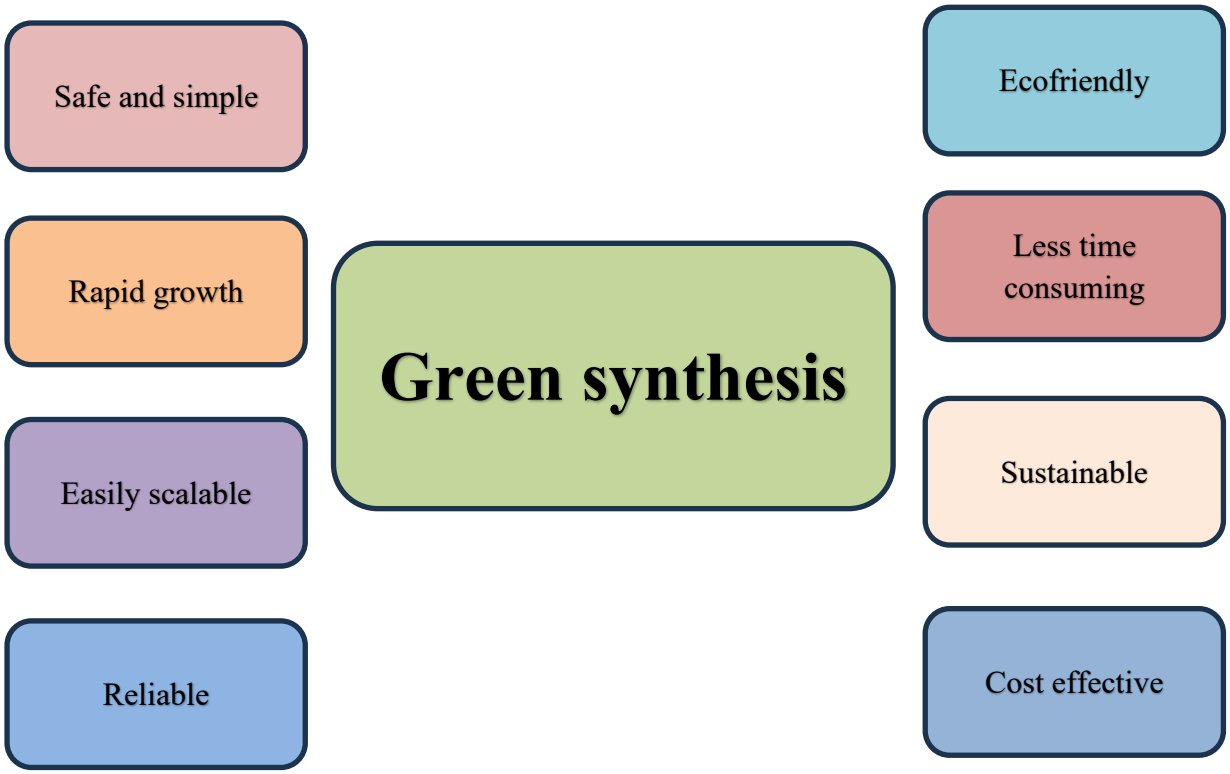


Figure 1.3 Advantages of green synthesis (Kumar et al., 2017)

As a way of green approach various microorganism mediated syntheses done by bacteria, fungi, and plants because of their reducing and capping ability to transform metal compound into reduced nanoparticles. Also cellulosic materials, algae, yeast used synthesize various nanoparticles as a way to approach green synthesis. (Kharissova et al., 2019; Ortega-Arroyo et al., 2013; Pal et al., 2019; Rafique et al., 2017; Rauwel et al., 2015; Sathishkumar et al., 2009; Yang et al., 2010).

Microbes mediated synthesis has some disadvantages such as time consuming, not so cost effective, and also not easy to handle. (Mittal et al., 2013). Plant extract mediated synthesis depends on some factors. The yield of product and reaction rate of synthesis depends upon the phytochemicals present in the plant. Sometime yield decreases due to absent of major phytochemical and also lower secretion, because the rate of secretion is always not the same. Fabrication of mono dispersed and control sized nanoparticles cannot be executed. Though plant mediated synthesis also have some limitation among all these green materials. Synthesis via plants parts is less-time consuming, rapid, and growth, availability of raw materials and also cost effective. (Ajitha et al., 2015)

Table 1.2. Comparative data of various green materials

Green materials	Process	Cons
Plant	Rapid synthesis and reliable size can be gain by changing parameters e.g. temperature, pH. Raw materials availability, cost effective.	Capping agent (reactive compound) cannot be determined easily.
Bacteria	Safer	Broad particle size distribution and large particle formation Various organic solvents needed to synthesize pure nanoparticles. Slower process.
Fungi	Smaller particles gained than the bacterial mediated synthesis. Affordable, easy to handle	Broad particle size distribution and also organic solvent needed
Enzyme	Enzymes can be found in pure from, purification of nanoparticles is easy.	Extraction of enzymes is time consuming and some enzymes are expensive.
Bio-polymer	Nanoparticles with better properties	Reducing capacity is nit fixed

Different parts of plants such as leaf, roots, stem, fruit, flower, and rhizome are used for plant-mediated synthesis of nanoparticles. (Jagtap & Bapat, 2013; Karthiga, 2018; Samreen et al., 2018).

Above all, extract of various parts of the plant used for synthesis procedure, because plants extract contains various phytochemical which act as a capping agent. (Ahmed et al., 2015).

Plant extract consist of various metabolites e.g. alkaloids, terpenoids, amino acids, alcoholic compound, flavones, flavonoids, proteins, polysaccharides, and phenolic compound are very much responsible for reducing Ag ions into nanoparticle. But the mechanism is very unknown of the plant mediated synthesis of nanoparticles. (Krishnaraj et al., 2010). Some of the phytochemical present in plants act as capping agent and stabilizing agent also.

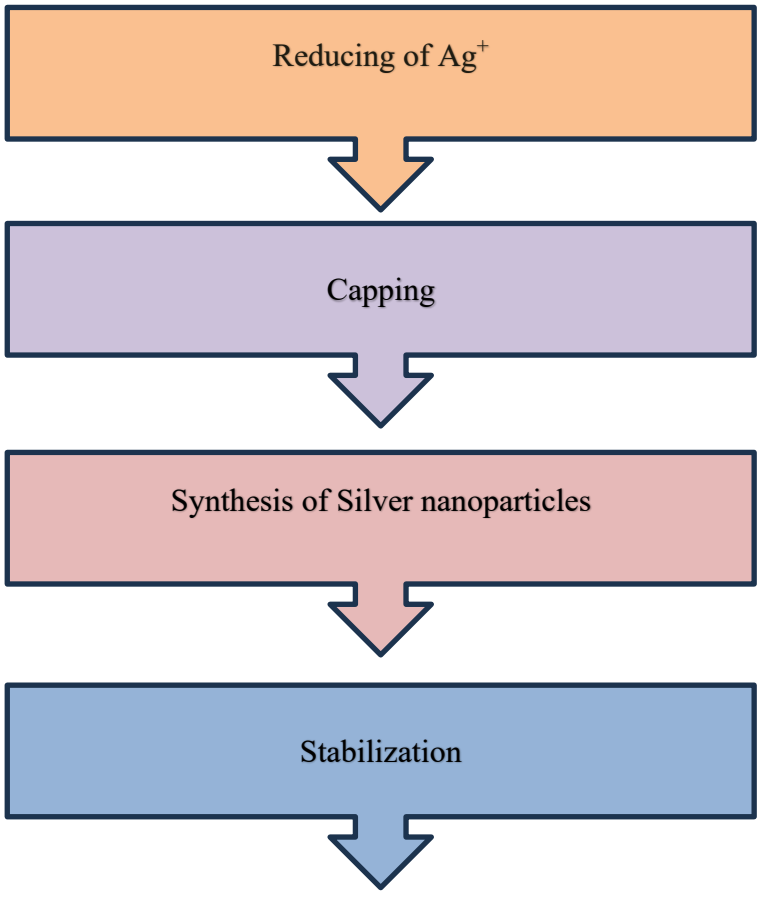


Figure 1.4 Working mechanism phytochemicals in synthesis of AgNPs

Lantana camara (**common lantana**) is a species of flowering plant which belongs to family Verbenaceae and is a genus of about 150 species of herbs. (G. C. Negi et al., 2019).

Figure 1.5 Plant *Lantana camara*

Lantana is a native spicies to tropical and subtropical america by origin. And introduced as ornamental and hedge plant to other countries in the world. It spreaded in to various areas ranging from open, unshaded areas, such as corp fields , to distributed areas as roadsides, railway tracks and and hillside. (Kato-Noguchi & Kurniadie, 2021).

Lantana, as described by Linnaeus in 1753 in Species Plantarum contains seven species, six from South America and one from Ethiopia. Being as species in tropical and sub-tropical region of America few taxa homegrown to tropical Asia and Africa. Carrying hundreds of cultivar various species being cultivated for its flowers in many countries where it occurs in approximately over 50 countries. 150 species of it is a better estimate where 50 to 270 specific and sub-specific entities out there. (G. C. S. Negi et al., 2019).

Lantana is a perennial shrub 1–4 m tall and forms dense stands. The leaves are opposite with long petioles, oval blades, hairy, and serrate. The species flowers all year round if the condition is adequate. (Kato-Noguchi & Kurniadie, 2021)

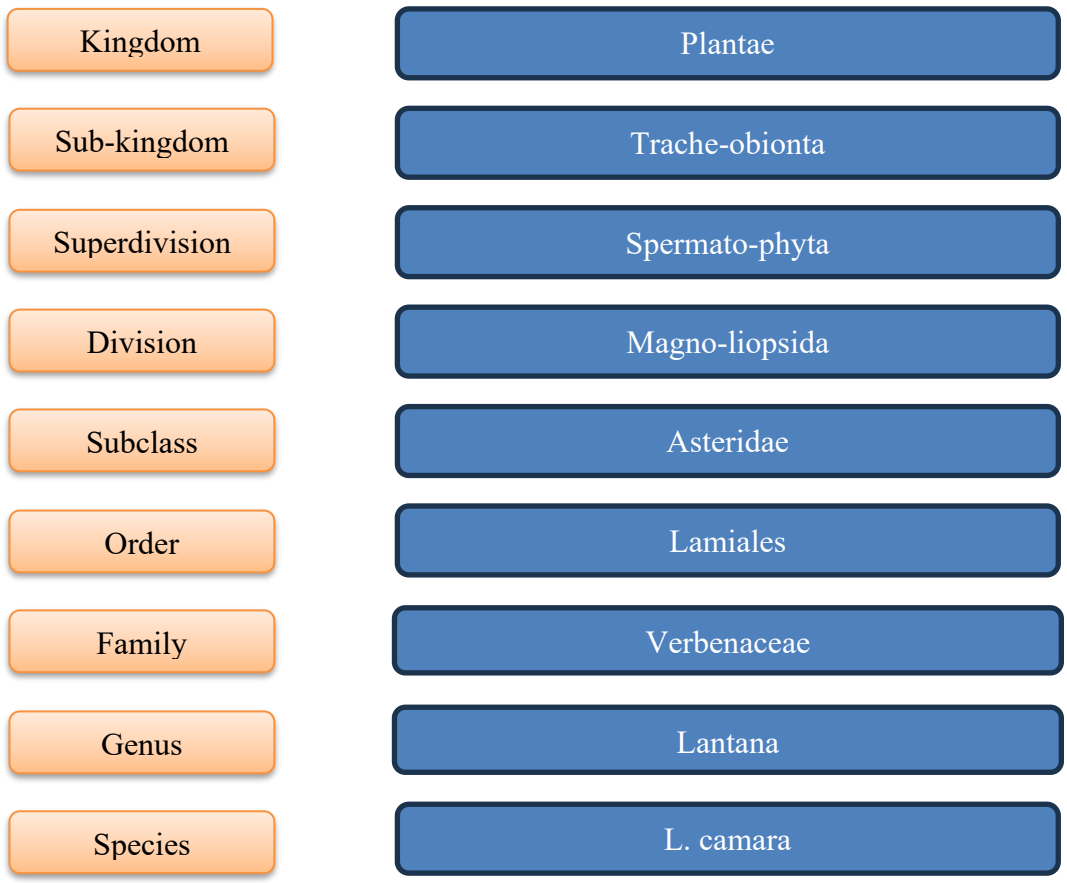


Figure 1.6 Taxonomic hierarchy of *Lantana camara*. (Ved et al., 2018)

Table 1.3. Phytochemical Analysis of *Lantana camara*

Phytochemical	**Result**	**References**
Phenoic compounds	(+)	
Flavonoids	(+)	(Kalita et al., 2011)
Glycosides	(+)	
Phytosterols	(+)	
Saponins	(+)	
Alkaloids	(+)	
Proteins	(+)	
Oil and fats	(-)	
Tannins	(-)	
Charbohydrades	(-)	

Positive sign indicates the presence where negative sign indicates the absence

1.2 RESEARCH GAP

Significant research has been conducted on the synthesis of silver nanoparticles using different plant extracts for their potential applications. However, there remains a notable research gap concerning the synthesis of silver nanoparticles using aqueous leaf extract of *Lantana camara* and the assessment of their photocatalytic activity. This particular gap in the literature requires investigation to explore the unique properties, stability, and efficiency of silver nanoparticles synthesized through this method, and to ascertain their potential in photocatalytic applications, which could have significant implications in environmental remediation and advanced materials science.

1.3 OBJECTIVES OF THIS PRESENT STUDY

The objectives of this present study are summarized below:

To develop a robust and reliable method for the synthesis of silver nanoparticles using the plant extract of *Lantana camara.*

To characterize the synthesized silver nanoparticles in standings of their size, shape, composition, and stability.

To assess the photocatalytic activity of the *Lantana camara*-synthesized silver nanoparticles in degrading or detoxifying a selected target pollutant or organic compound. And determine the optimal conditions and parameters for maximizing the photocatalytic efficiency of the synthesized silver nanoparticles.

To evaluate the potential environmental and industrial applications of Lantana camara-synthesized silver nanoparticles for photocatalytic purposes.

Chapter 2: LITERATURE REVIEW

2.1 REVIEW ON NANOTECHNOLOGY

At the beginning of 16th century nanotechnology was employed unknowingly to construct Damascus swords and other products till now. Nanotechnology is a branch of nanoscience where materials in the nanoscale exhibit significant and novel improved physical, biological and chemical properties in the field of science (Daniel & Astruc, 2004). In nanoscale nano meter stands for (10^{-9} m). A novel material in the field of nanotechnology is nanoparticle, nanoparticles show distinct properties such as, thermal, electrical, chemical, mechanical, magnetic, physical, optical and biological properties (Daniel & Astruc, 2004; Schmid, 1992). These properties of nanoparticles differ very significantly due to their reduced size from those of bulk materials. Having a large surface energy, high surface to volume ratio and reduced imperfections nanoparticles exhibit these unique properties in the field of application in various sectors. There are some excellent advantages of nanoparticles over bulk materials include Surface Plasmon Resonance (SPR), enhanced Rayleigh scattering, and semiconductors quantum size effects. As a result, nanotechnology is considered instrumental in achieving a clean and sustainable future. Nanoparticles are also regarded as the fundamental building blocks for the next generation of optoelectronics, electronics, medicine, catalysis and diverse chemical and biochemical sensors (Salamanca-Buentello et al., 2005)

Synthesis approaches: Top-down and Bottom-up methods (Wong & Schwaneberg, 2003). Top-down approach, a solid material abridged to nanoscale dimensions through physical processes like milling and attrition. One of the main drawbacks of the topdown synthesis method is that the size, shape and geometry of nanoparticles cannot be controlled through this synthesis procedure. The other

approach Bottom-up method which creates nanoparticles by considering atoms or molecules, which is consequently very versatile because the shape and size of nanoparticles can be achieved through this synthesis procedure. That's why compared to bottom-up approach top-down synthesis methods are not available to produce all kinds of shapes and geometries of interest. There are various characterization methods with their major development through this perspective exceeding physical and chemical characterization. Physical characterization as structural characterization can be done using various methods. SEM is the most widely used characterization technique for determining the structure and surface of nanomaterials (Pawley, 1997). SEM can operate at various magnifications from 10 up to 300k and can exhibit chemical composition of the surface. FE-SEM is the advanced version of SEM where images can be obtained with more clearance and less electro statical distortion (Murat, 1975; Pawley, 1997). By XRD analysis the crystalline nature, structure, size and lattice constant can be obtained of nanoparticles (Kohra & Kikuta, 1967). TEM can give both image and diffraction information with magnification from 50 to 106 with negligible electro-charging of samples using low voltage (Konno et al., 2006).

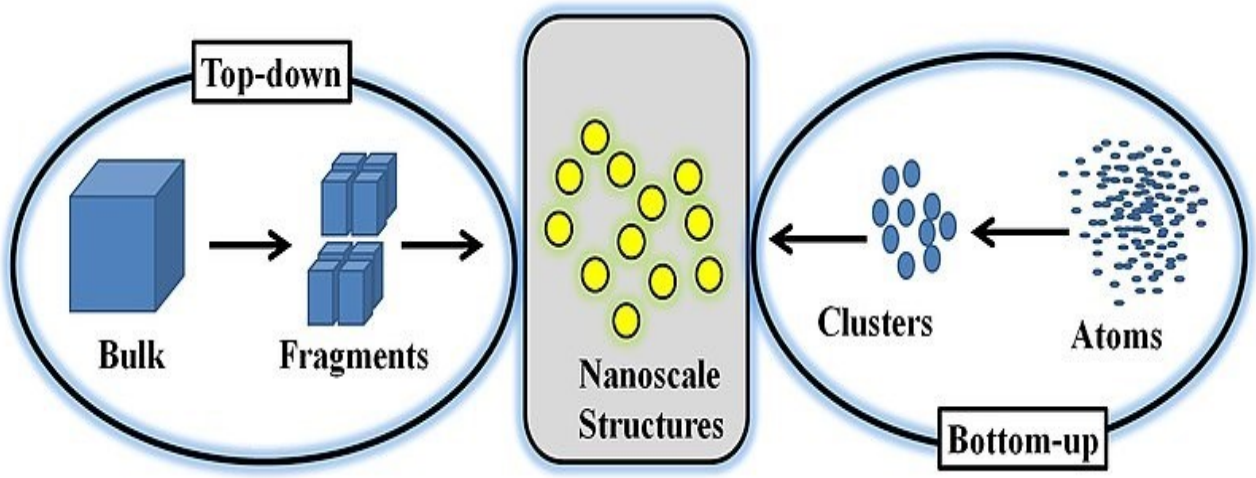


Figure 2.1 Top Down and Bottom Up approaches of nanoparticles synthesis

Ther is a new characterization technique which can give three dimensional (3D) real space images of nanomaterials named Scanning Prove Microscope (SPM) (Faraday, 1857).

For chemical characterization there are some techniques such as optical, electron, and ionic spectroscopy to evaluate the surface and interior atoms and compounds and their spatial distributions. To ascertain the electronic structures of nanomaterials, various techniques such as optical microscopy, absorption and transmission spectroscopy, photoluminescence, infrared, and energy dispersive X-ray (EDS) spectroscopy are utilized. Specially (EDS) and (XPS) used to determine chemical configuration by X-ray and auger electrons emitted by a material (Liu et al., 2004).

2.2 REVIEW ON NANOPARTICLES

Nanomaterials have been existing for a very long time thousands of yeas. Many ancietnt civiliozaiton emploeyd gold, silver, platinum and palladium nanoparticles in pants more then 3000 years ago, but they don't have proper knowledge about their existance or unique properties (Ramanavicius et al., 2005). Due to having lack of knowledge and absance of analytical methods to investiagte materials smaller then 1 μm many infromation about these materials remain limited until the last century. Methods TEM and SEM allowed for visualization on the scale (10^{-9} = 1 nm) and revealed the szie range of molecules and atoms. So following this breakthrough in microscopy laid the foundation for the developmoent of nanotechnology in the early 20^{th} century (Konno et al., 2006). Over the past 50 years, several new nanomaterials have been discovered which have intriguing physical properties and some of them have been recognized with Novel prizes (Novoselov et al., 2005).

In general, nanoparticles are described as particles with at least one dimension within the nanoscale range of 1-100 nm. On the other hand, nanomaterial possess internal or external structures within the nanoscale. In the fabrication of nanostructures nanoparticles serves as the fundamental building block. They exist on a scale larger than individual atoms or simple molecules governed by quantum mechanics. Nanoparticles involves considering not only their size also their other aspects, as also shape and structure in figure (Borm et al., 2006). Both size and composition influence major properties of nanoparticles as physical and chemical properties. Nanoparticles can consist of organic and inorganic materials where organic materials include nano-polymers, bucky ball and inorganic materials include metals, metal oxide, metalloids etc. (Ahamed et al., 2015). Depending on these factors the properties of nanoparticles are diverse in a wide range which makes them highly versatile and attractive for numerous applications.

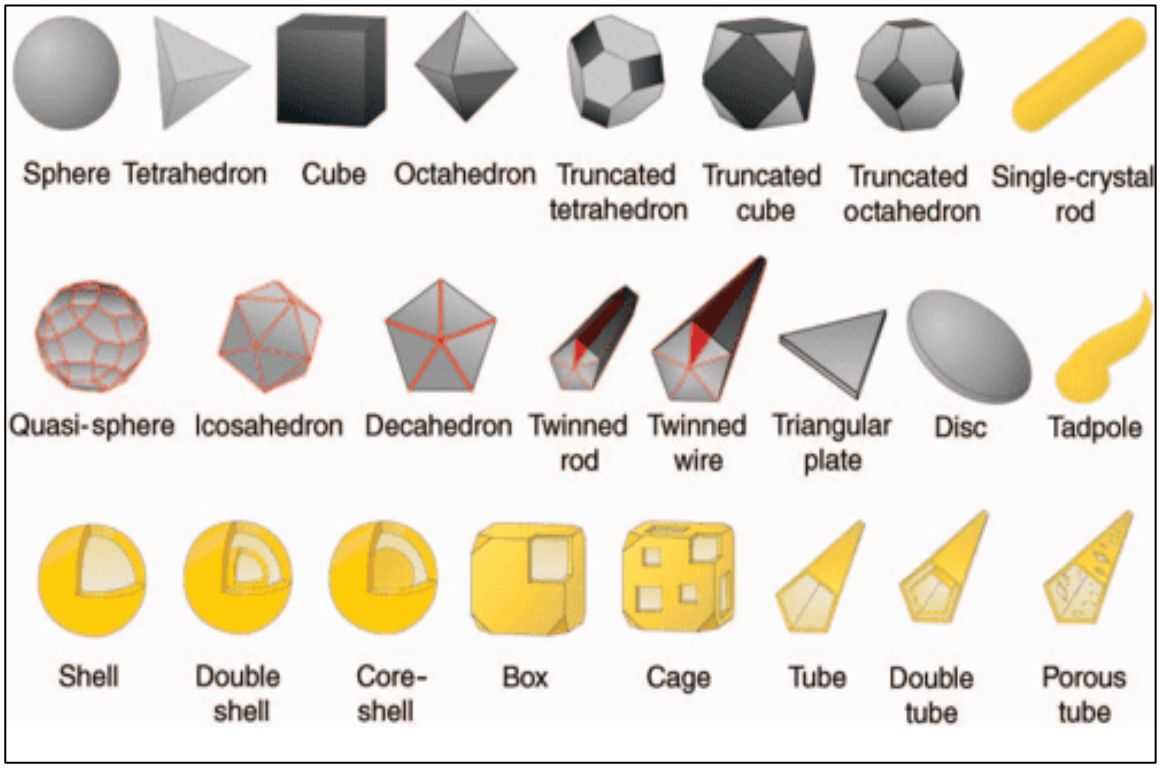


Figure 2.2 Different structure of nanomaterials

Different properties of the materials such as catalytic activity inactivity especially photocatalytic activity, depend on the surface nature of nanoparticles (Kettler et al., 2014). There is high interest in improving the surface of nanoparticles to obtain the desired material properties by optimization or other modification because of the importance of nanoparticles surface nature. This modification of nanoparticles can be achieved by adding different types of compounds as an additive (Kettler et al., 2014). These are used to prevent the agglomeration, and various other properties (Pautler & Brenner, 2010). By modifying nanomaterials enhanced catalytic activity of the material or other further features can be achieved, including organic dye degradation, antimicrobial and antifungal activities etc. (Sperling & Parak, 2010). In the last two decades, nanomaterials have gained so much interest in the applications in the area such as computing, sensors, biomedicine and other fields (Amin et al., 2015).

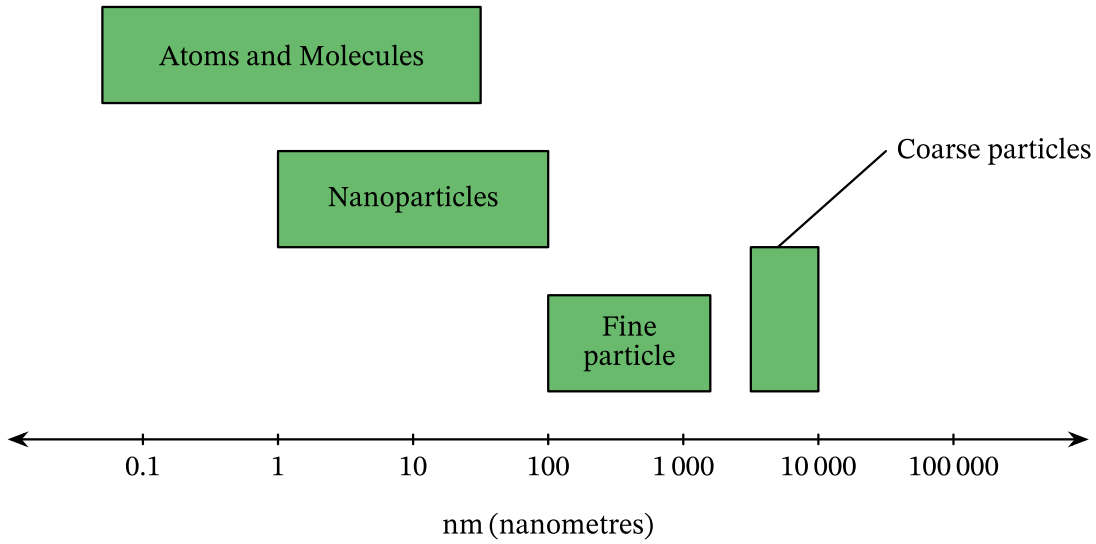


Figure 2.3 Nanoparticles size comparison

The both chemical and physical properties of metallic nanoparticles are diverse from bulk materials as lower melting points, specific optical properties, higher surface area, and mechanical strength. There are several types of classification for nanoparticles reported in many literatures and these can be based on shape or materials. In general nanomaterials can be classified by materials, mode of use (composite, individual particles etc.) or shape. There are two categories depending on the chemical nature of the nanoparticles: Organic nanoparticles include nanosized polymeric particles and carbon nanotubes and Inorganic nanoparticles include ceramic particles, and metallic particles. Three categories can be used to categorize nanoparticles in terms of dimensions: One dimensional system, (Thin films (size 1-100 nm) or monolayers gaining great importance because of their application in the different fields as biological and chemical sensors, optic and magneto-optic devices, and fiber optic systems. Second one is Two-dimension nanoaprrticel e.g., Carbon nanotubes, third one three dimension nanoparticles e.g., Dendrites, Quantum Dots QDs). By using various microscopic techniques size, surface morphology and surface charge of nanoaprticles characterized (Annabelle, 2004; Pokropivny et al., 2007).

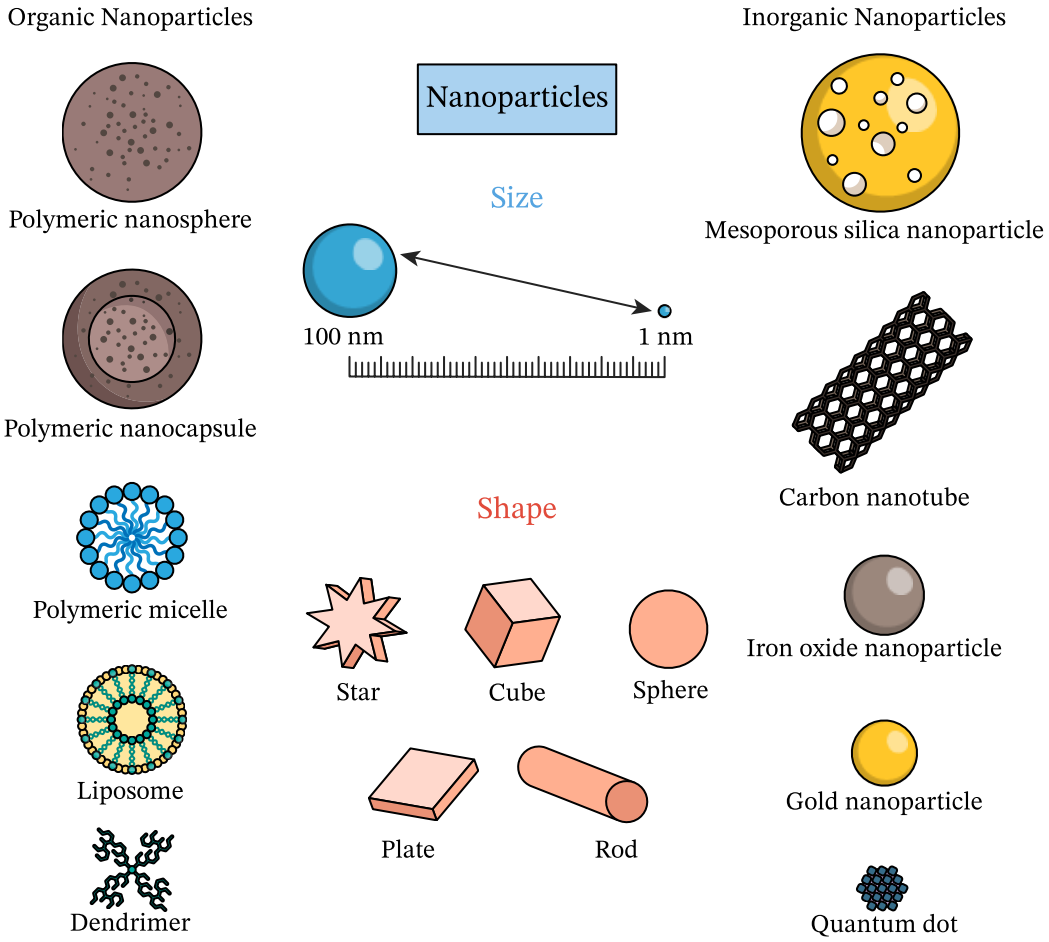


Figure 2.4 Different kinds of nanomaterials

2.2.1 Organic nanomaterials

Organic nanomaterials include carbon-based compounds such as carbon nanotube and nano-sized polymeric materials. Being a complimentary one to inorganic nanomaterial organic nanomaterials contain unique properties like hogh reaction activity, optoelectronic properties, good processibility (Balakrishnan et al., 2006), high photoluminescence (PL) efficiency (An et al., 2002) and good doping properties (Peng et al., 2005). There are many conventional methods out there for oraganic nanomaterials synthesis as

reprecipitation (Briseno et al., 2007), laser irradiation (Sperling & Parak, 2010) and microemulsion (Debuigne et al., 2000). Organic nanomaterials have been fabricated into differents shapes like nanotubes (Zhou et al., 2010), nano pallets, nanoparticles (Zhang et al., 2007), nanobelts (Balakrishnan et al., 2006), nanowires and nanoribbons. One dimensional (1D) organic nanomaterials have been especially accentuated. 1D organic nanostructures have a broad and wide range of applications in various sectors like lasers, sensors, color-tunable display and field-effect transistors (Briseno et al., 2007). Carbon nanotubes one of the important member of organic nanomaterials containing carbons in their structure. There are two types of carbon nanotubes such as Single wall (SWCNT) and multi wall (MWCNT) carbon nanotubes. There are many synthesis aproaches for synthesis of carbon nanotubes includidng various physical and chemical methods as laser ablation (Shen et al., 2005), arc-discharge (O'carroll et al., 2007), chemical vapor deposition (CVD) (Nikolaev et al., 1999), and solvothermal reduciton (Shen et al., 2005). Depending on their helicity and diameter carbon nanotubes have many physical and chemical properties and application in the field emission, hydrogen storage and solar cells (Dervishi et al., 2009).

2.2.2 Inorganic nanomaterials

In the field of energy application materials that contain strong electrical, optical, mechanical and thermal capabilities are best candidiates. Inotganic nanomaterials gaining interest for use in enegry applications. To meet the demands of various energy applications inorganic nanopartilces undergone substabtial research with multifunctionalization. Containing special properties such as good electrical and thermal conductivity, wide surface areas, and chemical satbility, inorganic nanoparticles plays a important part in energy generation, energy conversion, energy storage and energy transmission

applications (Wang et al., 2020). Inorganic nanoparticles include metallic nanoparticles, metal oxide nanoparticles or ceramic nanoparticles.

2.2.3 Metallic Nanoparticles

Metallic nanoparticles have opened many new pathways with their unique properties in the field of nanotechnology and nanoscience. Metallic nanoparticles have some special properties with containing appropriate functional groups in their structure and they can be modified and synthesized through various procedure which make them vulnerable to bind with ligands, antibodies and drugs (Prasad et al., 2013).

Metallic nanoparticles refer nanosized metals with the size range between 10 nm -100 nm or at least one dimension in nanoscale. Surface plasmon resonance (SPR) one of the very unique properties of metallic nanoparticles. Among the others novel metals, especially silver and gold, have gained interest in the field of research such as catalysis, medical etc. The most recently discovered characteristic of nanoparticles is their extremely high surface to volume ratio and their universal ability to interact with other particles. Its large surface area to volume ratio accelerates the diffusion of nanoparticles and improves their practicality at low temperatures. Without harming or poisoning of healthy cells affected cells and tissues can be treated. Metallic nanoparticle also show very good optical properties due to SPR band resonance (Kumar et al., 2018).

2.2.4 Silver Nanoparticles

Among other metals, silver nanoparticles (AgNPs) gaining great importance day by day due having some unique properties like localized plasmon resonance and antimicrobial properties (Franci et al., 2015; Jana & Pal, 2007; Stiufiuc et al., 2013), chemical/biological sensors and biomedicine materials (Alon et al., 2014; Evtugyn et al., 2014; Thanh & Green, 2010), biomarker (Bu & Lee, 2012; Luo et al., 2015; Rivero et al., 2013), and so on. Silver nanoparticles have size range between 1-100 nm. Having some unique properties nanoparticles are

emphasized into various industrial application of catalysis, electronic and photonics. In recent years with the increasing usage of electronic machineries, with utilizing the space between these circuits creating a interest for the thickness (Iwama & Sahashi, 1980; Sondi et al., 2003; Tsuji et al., 2002). So far, numerous techniques and strategies, including chemical, photochemical, physical, and biological processes, have been described for the development of silver nanoparticles. Every method and approaches have merites and demerites such as costly, particles size, size distribution and scalability and so on (Lee et al., 2005; Lennon et al., 2007; Smetana et al., 2005; Wakuda et al., 2008). Major drawbacks of photochemical and physical method are they need very high temperature, expensive equipment and need high vacuum condition (Iravani et al., 2014; Magnusson et al., 1999; Toisawa et al., 2010). Considering these problems green approach gained very much importance in the research field. Water in green approaches, an eco-friendly reagent, and free from any kind of toxic materials and reagents for the stabilizing nanoparticles.

2.3 REVIEW ON SYNTHESIS METHODS OF SILVER NANOPARTICLES

2.3.1 Physical Method

Physical approaches, following the method evaporation condensation metal nanoparticles synthesized. A carrier gas carries vaporized source materials inside the furnace. Various kind of nanoparticles such as gold, silver, fullerene and PbS previously synthesized using condensation and evaporation mechanism (Gurav et al., 1994; Kruis et al., 2000; Magnusson et al., 1999).

Some disadvantages of synthesis of AgNPs using a tube furnace such as required huge amount of energy, take a lot of time, and occupies a large space. Laser ablation method employed to synthesize AgNPs from solution which contains bulk materials (Iijima, 1991; Mafuné et al., 2000; Sylvestre et al., 2004). Pure colloids gained in this method for further applications(Tsuji et al., 2002). Hence

physical approaches of synthesis of AgNPs usually considered physical energy to produce nanoparticles and resultant narrow size distribution.

2.3.2 Photochemical Method

AgNPs very firstly synthesized by Huang and Yang via photoreduction method from inorganic clay suspensions $AgNO_3$ where it was present as a agent helps in stabilization and prevents aggregation of nanoparticles. For achieve a relatively stable size with single mode of distribution, AgNPs fragmented into smaller size by irradiation R. Expensive equipment and experimental environment required in this method which is a major drawback.

2.3.3 Biological Method

Over the various complex synthetic procedure biomolecule mediated synthesis is a great approach toward green chemistry which is a very simple and viable alternative method. Biomolecule like polysaccharides, biological microorganism present in various biological sources as bacteria, fungus or plant extract act as reducing agent for the synthesis procedure. Bacteria are known as bio-factories for synthesis of novel nanoparticles (Ag, Au) and also produces inorganic materials either intra- or extracellular. Previous studies reported biosynthesis of nanoparticles like using tea (Camellia sinensis) extract to synthesis of gold silver nanoparticles in aqueous solution. Bio-organisms act as nonpathogenic bacterium and synthesized AgNPs become highly stable. As an ecofriendly approach biological methods have many advantages over any other conventional chemical methods for AgNPs synthesis and as well as cost effective technique.

2.3.4 Chemical Method

Chemical reduction is on of the mostly used method for the synthesis of nanoparticles because of its easily experimental procedure and simple equipment. Silver nanoparticles can be synthesized via various chemical

methods with low cost and high rate of yield. Silver nanoparticles can be chemically produced in solution by taking into account three essential components: Metal precursors, reducing agents, and stabilizing/capping agents are the first three. Nucleation and subsequent growth, two stages followed by reduction of silver salts execute the formation of colloidal solutions oof nanoparticles. Size and shape of nanoparticles depend, if all the nuclei form in the same time then they will exhibit similar size and have the same successive growth rate with mono-dispersity among them. By regulating the reaction's many components, including the precursors, pH, temperature, and reducing agents (such as sodium oleate and PVA).

2.4 REVIEW ON GREEN SYNTHESIS METHODS OF SILVER NANOPARTICLES

Silver nanoparticles have gained great attraction among the researcher in recent years because of having wide range of applications such activity against various microorganisms and also conquering drug resistance against commonly used antibiotics (Sharma et al., 2009). Due to having special characteristics AgNPs can be applicable in various fields like drug delivery (Prow et al., 2011), biomedicine (Chaloupka et al., 2010), water treatment, agricultural (Nair et al., 2010) etc. AgNPs have been synthesized via various conventional methods such as laser ablation (Abid et al., 2002), microwave (A. Khan et al., 2011), autoclave (Yang & Pan, 2012), chemical reduction (Z. Khan et al., 2011) etc. These methods have some major drawbacks as high operational cost, energy and contains harmful toxic chemicals though they have effective yield rate from synthesis.

Overcoming these issues of physio-chemical methods, there is need of cost free and energy efficient method for synthesis of AgNPs using various biomolecule present in microorganism (Sharma et al., 2009), plant extracts (Song & Kim, 2009), and natural polymers (Huang & Yang, 2004) where biomolecule play role as reducing agent and capping agent. Various synthesis of AgNPs focusing the

green method have been published previously (Gopinath et al., 2013; Mittal et al., 2013; Nangare & Patil, 2020; Prabhu & Poulose, 2012; Srikar et al., 2016).

2.4.1 Green Synthesis

The two primary components of the green synthesis technique are organic agents and metal ion solution, sometimes known as metal salt. reducing agent that is found in the cells and serves as a capping and stabilizing agent. No further stabilizing or capping agent needs to be added.

2.4.2 Metal-ion Solution

Silver ions are present in the solution of various silver salts as Ag^{+}, preliminary component for the synthesis nanoparticles. Among the various salts of silver, $AgNO_3$ is mostly used by researcher that contains Ag^{+} ion, with various concentration range from 0.1 mM to 10 mM for the synthesis.

2.4.3 Reducing Agents (Biological)

Biological reducing agents present in various biological sources distributed in nature. Four kingdoms out of five kingdom of living organism being used to synthesize AgNPs those contains various organism, hence four kingdoms: Monera (prokaryotic organism), Protista (unicellular organism), Fungi (eukaryotic), Plantae (eukaryotic, autotrophs) and animalia etc.

The green synthesis of silver nanoparticles mediated by biomolecules has been achieved by the utilization of various biopolymers, microbial cell biomass, plant extracts, and cell-free growing medium.

Plant-mediated synthesis of AgNPs is carried out by a variety of plants, including algae and angiosperms. For synthesis, a variety of plant components, including leaves, bark, roots, and stems, have been employed. With a few exceptions (Rao et al., 2014), plant extracts serve as both a capping and stabilizing agent in plant-mediated green synthesis. These chemical agents are employed to stabilize

AgNPs. Metabolites present in the plant proteins (Elumalai et al., 2014), and chlorophyll acting as capping agents for synthesis of AgNPs.

Water is one of the common solvents for the preparation of plant extract but there are also few studies concerning the use of various organic solvents like ethanol (Kulkarni et al., 2012; Logeswari et al., 2015), methanol (Rahimi-Nasrabadi et al., 2014; Sadeghi & Gholamhoseinpoor, 2015; Sadeghi et al., 2015) and ethyl acetate (Rajesh et al., 2012). Pretreatment process with saline or acetone (Mondal et al., 2014) (Bankar et al., 2010) atmosphere before preparation of plant extract mentioned by some other researcher. Bu in this case nanoparticle suspensions occurred only in aqueous medium only whatever the solvents used to prepare the plant extract. Comparing to synthesis of nanoparticles using bark, tissue and whole plant (Patete et al., 2011) plant extract facilitated synthesis exhibit nanoparticles with well define shape, structure and surface morphology.

There are certain disadvantages to using microorganisms to synthesize silver nanoparticles as opposed to biopolymers and plant extracts as capping and reducing agents, including slower growth rates, more culture upkeep, and more consistent inoculums. It is possible to successfully synthesis AgNPs by employing several fungal and bacterial species. There are two different ways to synthesise silver nanoparticles: one uses substances released in the growing medium, and the other uses the biomass of microbial cells directly. Either way can be used to synthesize silver nanoparticles. AgNP production occurs extracellularly, as documented in numerous research 5354, although it was also noted in a few earlier investigations (Mukherjee et al., 2001). One of the most widely used fungal genera for the synthesis of AgNPs is Fusarium (Ahmad et al., 2003; Ajitha et al., 2014b; Durán et al., 2005; Ingle et al., 2009). In this case, piperitone is used as a stabilizing agent in the synthesis, unlike Perni (Perni et al., 2014) and Shah-verdi (Shahverdi et al., 2007) fungi.

2.4.4 Mechanism of Silver Nanoparticles Synthesis

A variety of biomolecules found in biological sources are used in the green synthesis process to create AgNPs. Organic substances that provide electrons to the reduction of Ag+ ions to Ag0 include carbohydrates, fats, proteins, enzymes and coenzymes, phenols, flavonoids, alkaloids, gum, and so forth. Silver ion reduction is carried out by active moieties found in the utilized organism or extract. electrons produced when acids (ascorbic acid) and alcohols (catechol) in hydrophytes dehydrate, when keto is converted to enol in meso-phytes, or when both processes occur in xerophytes (Jha et al., 2009). Figure 2.5 is a schematic figure that illustrates the reduction of silver ions, agglomeration, and stabilization to generate AgNPs.

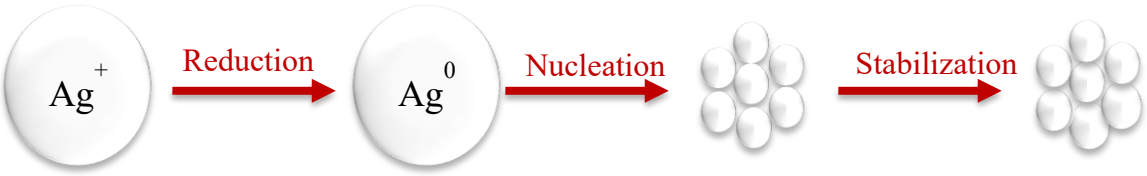


Figure 2.5 Synthesis scheme of AgNPs

2.4.5 Factors Affecting Silver Nanoparticles Synthesis

The production of AgNPs is disrupted by a number of chemical and physical variables, including pH, reaction temperature, duration, extract concentration, and metal ion concentration. The primary factors influencing the size, shape, and morphology of nanoparticles are extract content, metal ion concentration, and reaction time. Numerous scientists proposed a fundamental media for the production of nanoparticles (Roopan et al., 2013; Sadeghi & Gholamhoseinpoor, 2015; Yang & Li, 2013). Advantages reported for the synthesis of nanoparticles under basic medium goo yield, mono dispersity (Ashraf et al., 2013) and rapid growth (Das et al., 2012; Edison & Sethuraman, 2012; Khalil et al., 2014) with enhanced reduction process. Increasing pH level small and uniform nanoparticles can be achieved (Ashraf et al., 2013; Dehnavi et al., 2013; Dubey et al., 2010; Ortega-Arroyo et al., 2013; Sathishkumar et al., 2012). Reaction temperature and time of stirring are very important parameters among the others. There is a restricted temperature around 40 °C in mesophilic microorganism mediated synthesis while temperature marked up to 100 °C using biopolymers and plant extract reported by researchers. Temperature increasing (30°C - 90°C) resultant the increase of AgNPs synthesis rate 81. And also exhibit the smaller size AgNPs (Fayaz et al., 2009). It is reported from the previous study that size of the synthesized AgNPs lie below 50 nm using bryophytes, gymnosperm, algae and bio-polymers and size ranged between 100 nm while using angiosperm, algae and bacterial sources for synthesis. Synthesis of AgNPs using microorganism and bio-polymers with continues stirring conquer from agglomeration comparing to plants extract mediated synthesis. Synthesis rate or formation of AgNPs can be accelerated by smearing mechanical force externally during the synthesis.

2.5 REVIEW ON APPLICATIONS OF SILVER NANOPARTICLES

Due to having special characteristics and some unique properties AgNPs have gained great importance and potential for some biological applications. Some of these applications and their features are discussed

2.5.1 Anti-microbial Activity

Silver nanoparticles have exhibited excellent anti-microbial activity. Several methods and techniques have used by researcher to evaluate and examine the anti-microbial activity of AgNPs.

Disc diffusion or Well Diffusion Methods

The disc diffusion method is one of the most widely utilized techniques that many researchers have employed to assess the antibacterial activity of AgNPs in solution form. In short, this technique involves placing a uniformly sized disc on the surface of the targeted microbial immunized on the nutrient medium, then dipping the disc in progressively higher concentrations of AgNP. Anuj & Ishnava, 2013; Geethalakshmi & Sarada, 2013; Khalil et al., 2014; Kora et al., 2010; MubarakAli et al., 2011; Mukunthan et al., 2011) and well diffusion demonstrate the antimicrobial activity of nanomaterials as the inhibition zone increases around the disc (Ajitha et al., 2014a; Ashokkumar et al., 2015; Geethalakshmi & Sarada, 2013; Gopinath et al., 2012; Sathishkumar et al., 2012; Zhang et al., 2014). Rather than employing discs, the well diffusion method creates small-sized discs on the agar plate; these pits hold the test fluid. Nutrient plates are incubated under standard conditions in both approaches to produce a distinct inhibition zone. The diameter of the inhibition zone surrounding the well or disc reflects how effectively AgNPs work against the selected microorganisms.

2.5.2 Anti-bacterial Activity

AgNPs show antibacterial action against two types o bacteria, gram positive bacteria *Lactobacillus fermentum* (Zhang et al., 2014), *Streptomyces sp*. 83, *Bacillus cereus* (Deepak et al., 2011), *Brevibacterium casei* (Kalishwaralal et al., 2010), *S. aureus* , *B. licheniromis* (Kalimuthu et al., 2008) and gram negative bacteria, *E. coli* (Perni et al., 2014), *Entrobacteria* (Shahverdi et al., 2007), and *Ureibacillus thermo sphaerius* (Juibari et al., 2011).

AgNPs don't show same action and activity on bacterial strains (gram-positive & gram-negative) but they exhibit enhanced activity comparing to each other. Some researcher reported that bacteria like gram negative is more sensitive compared to gram positive bacteria (Gopinath et al., 2012; Zhang et al., 2013) where other researcher observed reverse results (Das et al., 2012; Khalil et al., 2014; Kora et al., 2010). AgNPs have a complex antibacterial action strategy which is not elucidated very well. Working mechanism explained as, antimicrobial action of AgNPs can be categories in two types: execution of inhibition zone and the bactericidal action. In the inhibitory action, the bacterial cells remain alive but their cell growth prohibited and in bactericidal action, bacteria cells perish due to action of AgNPs (Perni et al., 2014).

2.5.3 Anti-fungal Activity

AgNPs also show antifungal activity over various fungi (Bankar et al., 2010; Raut et al., 2014). Shape of AgNPs play a significant role in this case (Rai et al., 2012). Antifungal activity of AgNPs against *C. albanicans* species (Kim et al., 2009) attributed through inhibition of budding.

2.5.4 Anti-parasitic Action

AgNPs exhibit efficacious larvicidal properties against several vectors, including Aedes aegypt (Suresh et al., 2014), Culex quinquefasciatus (Mondal et al., 2014), A. subpictus (Santhoshkumar et al., 2011), A. subpictu (Santhoshkumar et al., 2011), and other parasites (Marimuthu et al., 2011). AgNPs' anti-parasitic

effect and mechanism are still unknown. However, larvicidal activity is carried out by denaturing organelles and enzymes that include sulfur-containing proteins and phosphorus-containing DNA (Gnanadesigan et al., 2011).

2.5.5 Other Applications

There are several applications of AgNPs, such as medicinal, therapeutic agents , as glycconano sensors for disease diagnosis (Sastry et al., 1997) and as nano carriers for drugs delivery (Cheng et al., 2014). Also there are many use of AgNPs as radiation therapy (Lu et al., 2012), in H_2O_2 sensor (Tagad et al., 2013), in ESR-Dosimetry (Guidelli et al., 2012), sensors for detecting heavy metals (Kirubaharan et al., 2012) and as catalyst for reduction of dyes (Edison & Sethuraman, 2012)

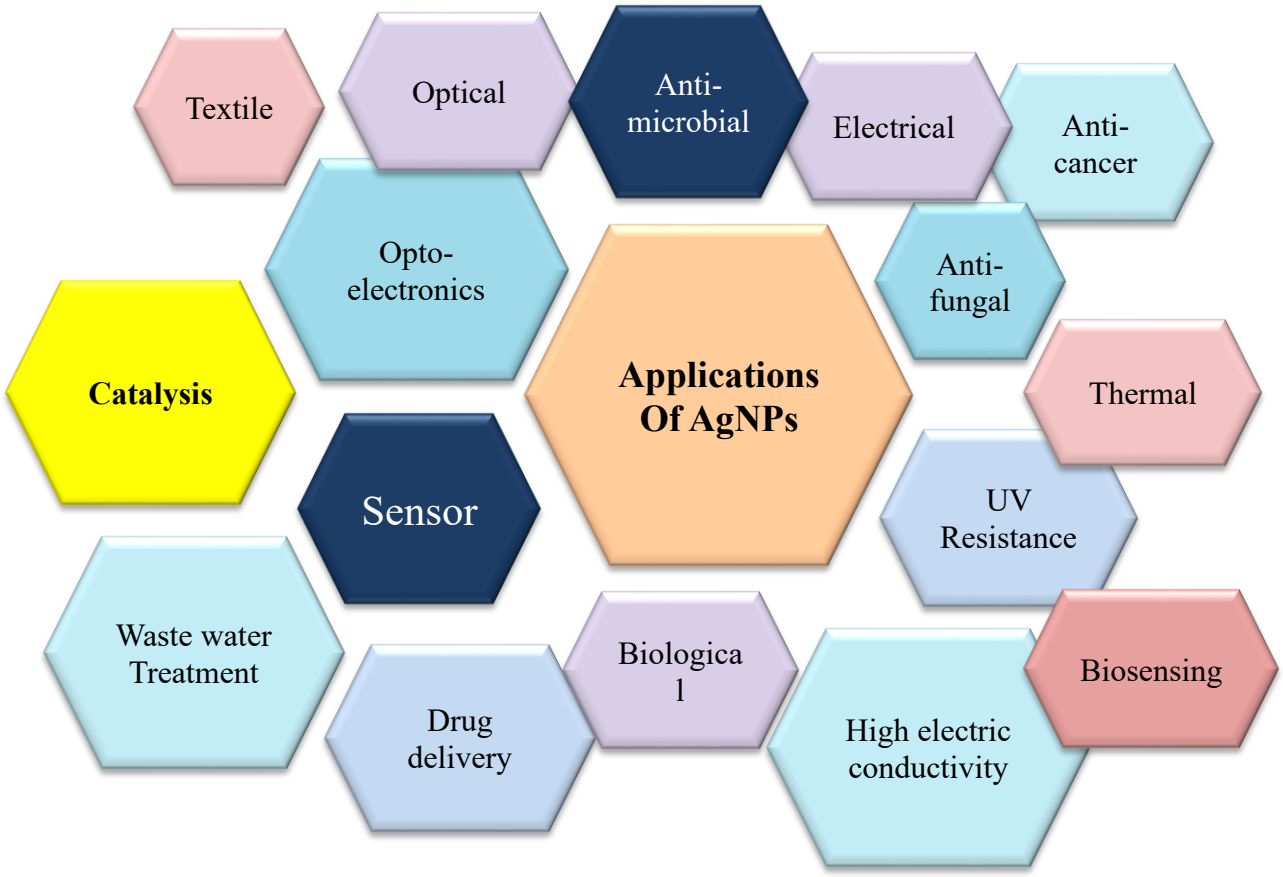


Figure 2.6 Various applications of AgNPs

2.6 REVIEW ON CATALYSIS PROPERTIES OF SILVER NANOPARTICLES

Organic dyes are spreading as their usage in everyday life with the increasing of population, industrialization and civilization. There are many sectors consisting organic dyes those are food industries, leather industries, pharmaceuticals, cosmetics, paints, plastics, paper and textiles. The major drawback is wastewater treatment process of these industries which contains various organic dyes included into post-production of products from these sectors. These organic dyes are very harmful for both human health and environment. There are various conventional and traditional color removal methods out there. Hence because of their stability due to having aromatic structure elimination of various dyes from water body is a problematic factor. In that case nanocatalyst plays an important role in reducing these synthetic dyes. There are many reports regarding photocatalytic degradation of methylene blue using biosynthesized silver nanoparticles under solar (sunlight) irradiation. One of the important factors in this process is exposure time or exposure to sunlight radiation. In briefly, physical appearance as color of methylene blue dye from deep blue to light blue or colorless after certain time of reaction. During the reaction procedure absorbance of dye will be measured via UV-Visible spectrophotometer at different time interval where absorption intensity decreases with the time passes. Decreasing rate of absorption intensity of dye indicates the degradation of dye in the presence of AgNPs. The degradation of dye depends on some factors such as concentration or amount of AgNPs catalyst, particle size, structure and morphology of nanoparticles. Particle size is one of the important factors among these which play a crucial role in the degradation process. Comparatively smaller particle with very high surface-to-volume ratio exhibits higher degradation rate. Is it known from precious reports that, AgNPs work as carrier of the electrons in the degradation process of dye.

Due to possessing a single exceptional property AgNPs exhibit photocatalytic activity when exposed to a light source through surface plasmon resonance (SPR), and their photonic excitation speeds up the pace of degradation. The dye degradation mechanism in the presence of solar light was depicted in Figure 2.7.

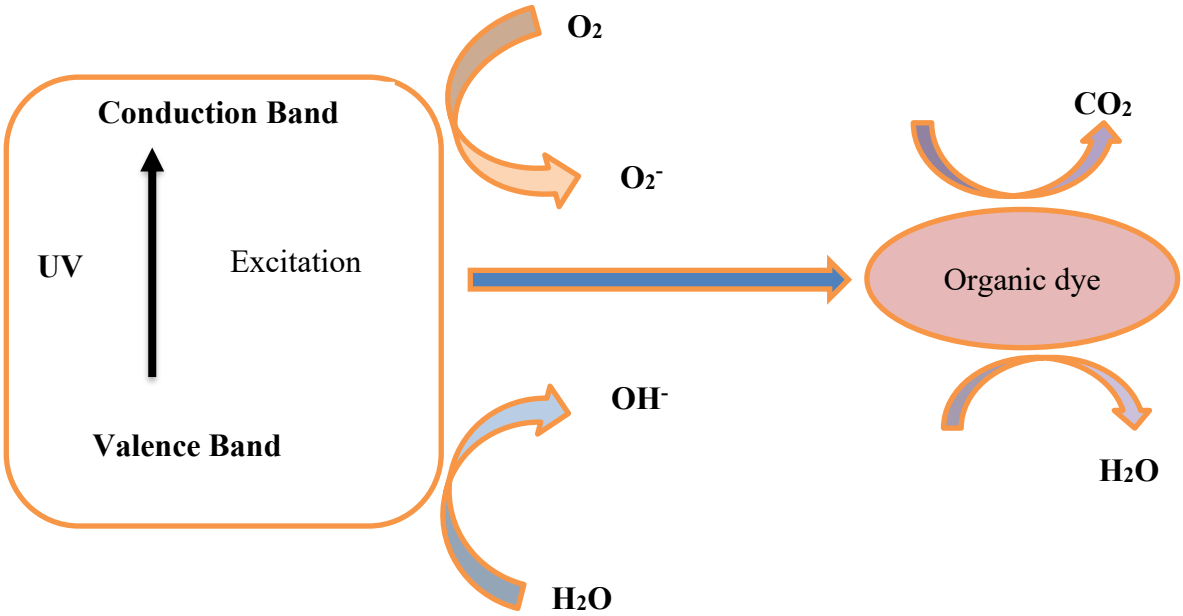


Figure 2.7 Mechanism of Dye degradation

Many studies reported on photocatalytic activity of biosynthesized AgNPs on different dyes, including Methylene Blue (MB) (Al-Zaban et al., 2021; Banu et al., 2021; Rohaizad et al., 2020; Saha et al., 2017), Rhodamine B (Awad et al., 2021; Wang et al., 2021), Nitrophenol (Princy & Gopinath, 2021; Seerangaraj et al., 2021; Zanjage & Khan, 2021), Congo Red (Zanjage & Khan, 2021), Textile Dye (Ahmed et al., 2020; Chand et al., 2021), Hexavalent Chromium (Lakra et al., 2021), Orange G (Baruah et al., 2019). Also biosynthesized silver nanoparticles are employed to reduce active pharmaceutical ingredients (APIs) like paracetamol (Al-Gharibi et al., 2021). And carcinogenic organic solvents such as benzene, toluene and phenol are also degrade using green synthesized silver nanoparticles (Ojha et al., 2021).

Chapter 3: METHODOLOGY

3.1 CHEMICALS AND REAGENTS

Silver nitrate ($AgNO_3$) (Merck KGaA, Germany), Deionized water (18 mili-Q), Methylene blue (PT. SMART LAB, INDONESIA), Dimethyl sulfoxide (DMSO), Mueller Hinton Agar (MHA) media (HIMDIA, India) and Potato Dextrose Agar (PDA), Ceftriaxone, Amphotericin B.

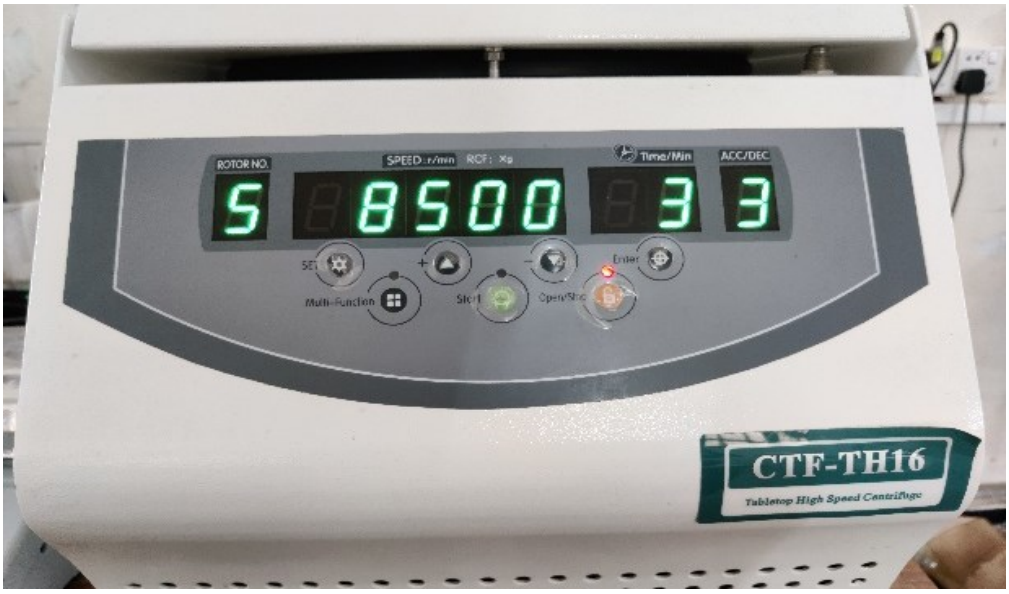
3.2 APPARATUS

For the synthesis, characterizations and the study of photocatalytic activity of AgNPs following instrument and apparatus were used.

Digital balance, Hotplate magnetic stirrer (**MS-H380-Pro, USA),** Centrifuge machine (CTF-TH16, China) Sonicator (Hwashin Powersonic 405, Korea), Oven, Vortex, UV-Vis spectrophotometer (T80+, UK), Scanning Electron Microscope (JEOL JSM-7600F), Energy Dispersive X-ray spectrophotometer, X-ray Diffraction Spectrophotometer (EMPREAN, Netherland)

UV-Visible Spectrophotometer

Oven



Centrifuge

De-ionized water source

Sonicator

Hotplate Magnetic Stirrer

Vortex

(Field Emission-Scanning Electron Microscope) FE-SEM

X-Ray Diffraction Spectrophotometer

Figure 3.1 Various apparatus used for this study

3.3 SYNTHESIS OF SILVER NANOPARTICLES

Approaching to an environment friendly process, aqueous leaf extract of *Lantana camara* was used as the reducing agent for the synthesis of silver nanoparticles (AgNPs) from $AgNO_3$ solution. The synthesis procedures as follows.

1) Collection of plant leaves
2) Drying
3) Grinding to make powder
4) Preparation of plant extract
5) Preparation of $AgNO_3$ solution
6) Synthesis of AgNPs
7) Purification
8) Solid particle

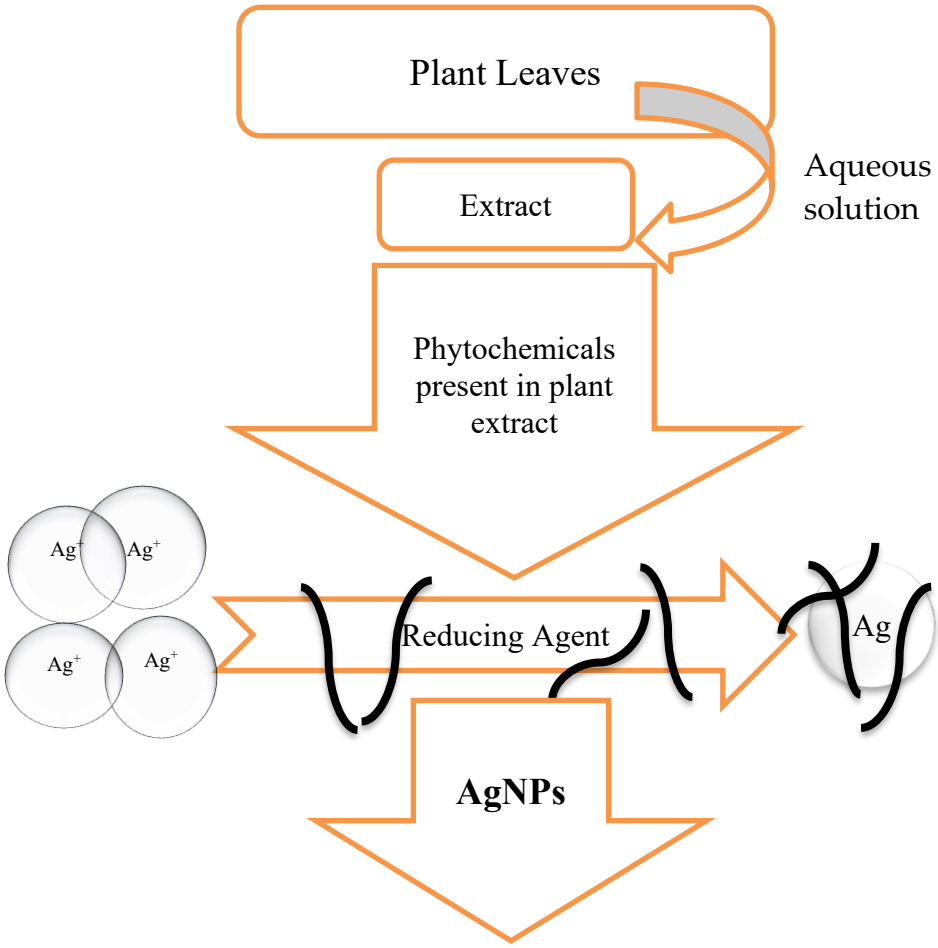


Figure 3.2 Schematic diagram of synthetic procedure

3.3.1 Collection of Plant Leaves

Fresh leaves of *Lantana camara* were collected from the area of Chittagong University of Engineering and Technology and others area of Chattogram hill sides. The plant is widely distributed in various areas of Chattogram and also in Bangladesh.

3.3.2 Sample Preparation

Fresh leaves of *Lantana camara* were washed throughly several times with the double distilled water to remove dirt and preventing any kind of contamination. Plant leaves were dried in shaded condition avoiding sunlight drying, because of metabolites present in the leaves may be affected by sunlight. After drying they were grinded to make powder and kept in moisture free condition.

Figure 3.3 *Lantana camara* leaves powder

3.3.3 Preparation of Plant Extract

10 g of leaf powder were added into 100 mL of Deionized water to make a concentration of 10%(w/v) and stirring at 60°C for 50 minutes. After cooling at room temperature, it is filtered through Whatman No 1 filter paper. A dark greenish-yellow color plant extract was obtained. Freshly prepared plant extract was used every time to synthesize AgNPs. To avoid any kind of contamination and precise result aseptic conditions were maintained properly throughout the whole procedure.

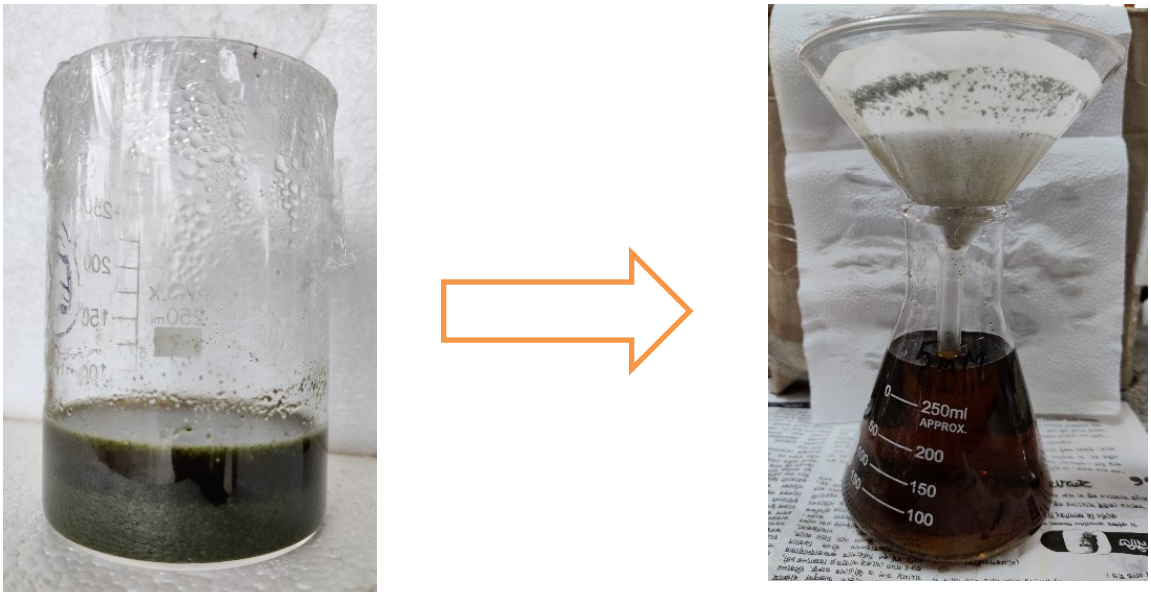


Figure 3.4 Aqueous leaf extract of *Lantana camara*

3.3.4 Synthesis of Silver Nanoparticles

In case of optimization of synthesis condition factors and parameters associated with the synthesis of AgNPs, it was subjected to many trials. Briefly, 5 mM of silver nitrate $AgNO_3$ aqueous solution prepared in a volumetric flask at room temperature used as stock solution, this solution prepared in the dark condition and kept in dark for further use. From that 100 mL of silver nitrate solution was taken in a conical flask and 10 mL (10% w/v) of plant extract was added into it slowly. For each synthesis 10:1 ratio of silver nitrate solution and plant extract was used. A hotplate magnetic stirrer was used to agitate the mixture for two hours at 70°C in order to reduce the silver ions to AgNPs and achieve full nucleation under dark conditions. and after that gave it a full day to finish the reduction process. It was noted that as the reaction continued, the solution's hue gradually altered. The solution was a paleyellow color at the beginning. The solution eventually turns dark brown, signifying the total reduction of silver ions and the creation of silver nanoparticles (Banne et al., 2017). Formation of AgNPs was confirmed by observing UV-Visible spectrum at range between 400-500 nm. The UV-Visible spectra were observed at the initial stage of the synthesis procedure till the reaction completed. After the synthesis procedure the colloidal mixture of silver nanoparticle was cooled at room temperature and centrifuged at 9000 rpm for several times followed by DI water washing for the purification of the particles and removal of unwanted organic compound. Centrifuging followed by ultrasonication for 5 minutes every time and then used vortex mixer to make the colloidal suspension dispersed homogenously. It was washed several times to remove unwanted organic moiety which can be present due to bio reduction by phytochemicals exist in leaf extract. After the centrifuge procedure separated AgNPs were collected and dried at 80°C for 2-3 hrs, and kept in vials in a moisture free condition. Figure 3.5 demonstrate the full synthesis procedure.

Figure 3.5 Synthesis and collection of AgNPs

3.4 CHARATERIZATION OF SILVER NANOPARTICLES

Synthesized silver nanoparticles were subjected to characterize through different characterization processes e.g. UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Field Emission-Scanning electron microscopy (FE-SEM), Energy dispersive X-ray analysis (EDS), X-Ray diffraction analysis (XRD). The structural morphology of nanoparticles was ensured by these methods.

3.4.1 UV-Visible Spectra Analysis

The formation of AgNPs by reduction of pure Ag^{+} was confirmed by observing UV-Visible spectra of reaction media from the very initial period at different time intervals, taking 3 mL of sample and compared with 3mL of De-ionized water used as a blank. The ultraviolet-visible spectrum of the synthesized AgNPs was record using UV-vis spectrophotometer in the range of wavelength between 400-500 nm. The baseline was adjusted with De-ionized water used as a reference blank (Shafaghat, 2015; Uddin et al., 2020).

3.4.2 Fourier Transform-Infrared (FT-IR) Spectroscopy Analysis

FT-IR analysis was done to elucidate functional groups present in the synthesize AgNPs with aqueous leaf extract of *Lantana camara.* The FT-IR analysis was performed by using the KBr pellet method in the wave number range between 500 cm^{-1} to 4000 cm^{-1}.

3.4.3 Field Emission-Scanning Electron Microscopy (FE-SEM) Analysis

The scanning electron microscopic analysis was done by Field Emission-Scanning Electron Microscope to identify the surface morphology and also the size and shape of AgNPs (Kabir et al., 2020).

3.4.4 Energy Dispersive X-ray (EDX) Analysis

The elemental signature of the synthesized AgNPs was analyzed by an Energy Dispersive X-Ray spectrophotometer. The operating condition of instrument was 10.0 kV acceleration voltage and 1.0000 nA emission current (Akter et al., 2020).

3.4.5 X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was done to adequate surface morphology of synthesized AgNPs and also acquire accurate information regarding the composition, crystal structure, and crystalline grain size of nanoparticles (Mody et al., 2010).

3.5 PHOTOCATALYTIC ACTIVITY OF SILVER NANOAPRTICLES

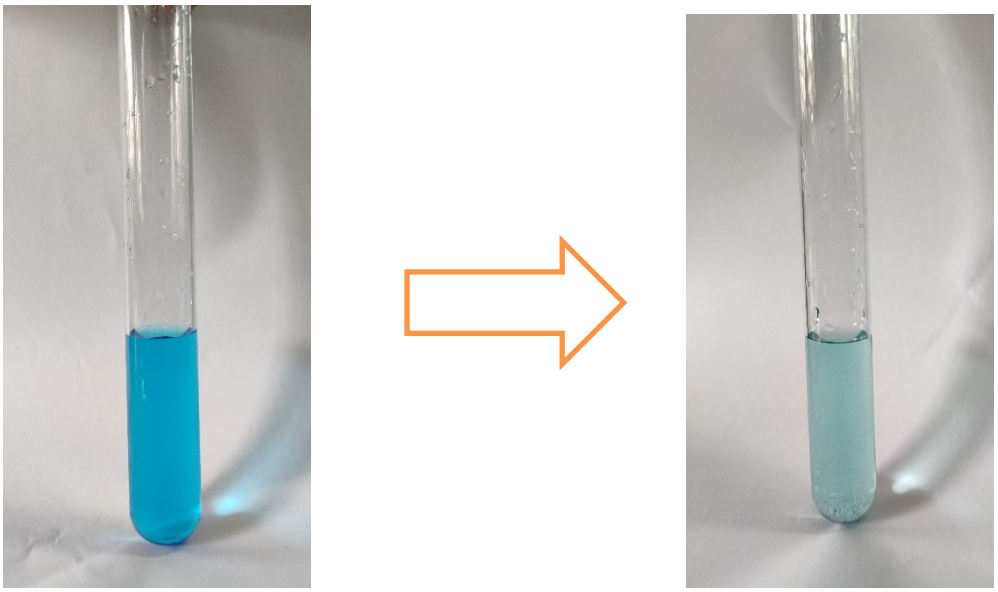
For evaluating the photocatalytic activity of silver nanoparticles degradation of a synthetic dye Methylene blue was examined under sunlight.

3.5.1 Preparation of Methylene Blue Solution

An aqueous solution of methylene blue was prepared in volumetric flask by adding 10mg of methylene blue in 1000ml De-ionized water and used it as stock solution, concentration of stock solution was 10mg/L and it further diluted to various concentrations by adding de-ionized water

3.5.2 Photocatalytic Degradation of Methylene Blue

The photocatalytic degradation of methylene blue by synthsized AgNPs was done. By taking 20 mL of methylene blue from the prepared stock solution and adding 125mg/L, 250mg/L and 500mg/L of AgNPs catalyst in individual methylene blue solution. They were kept under sunlight for the photocatalytic degradation of methylene blue dye with continuous stirring (Vanaja et al., 2014). Temperature and pH were at optimum range for the procedure. Different concentrations of methylene blue solutions were used for the procedure and whole experiment. The degradation of methylene blue was observed by taking absorbance at different time interval with de-ionized water used a reference blank by UV-Visible spectrophotometer. Measuring the absorbance of methylene blue in different time interval from initial up 120 minutes when the reaction media stands in phase equilibrium. Every time sample was taken to measure the absorbance of the MB dye through filtering it with Syringe filter to avoid particles when the absorbance taking for only methylene blue during the procedure. The appearance of methylene after 120 minutes of reaction shown in Figure 3.6.



Before Photocatalytic Reaction

After reaction

Figure 3.6 Color appearance of methylene blue before and after the photocatalytic reaction

The various parameters were investigated for the experiment as follows.

Table 3.1. Various investigated parameters for catalytic activity

Parameters	Investigate values
Catalyst dosage	125 mg, 250 mg, 500 mg
Concentration of MB dye	2.5 mgL^{-1}, 5 mgL^{-1},7.5 mgL^{-1},10 mgL^{-1}
Time	30 min, 60 min, 90 min, 120 min

3.6 CALCULATION OF DEGRADATION EFFICIENCY

Percentage of degradation was estimated by the following formula:

$$\text{Removal percentage (\%)} = (C_0-C_e)/C_0 \times 100$$

where,

C_0 = Initial concentration of methylene blue dye

C_e = Final concentration

3.7 MICROBIAL ACTIVITY ASSAY

The in vitro antibacterial activity of produced compounds was investigated using the Agar disc diffusion method (Balouiri et al., 2016). The basal medium employed for the study of bacterial and fungal strains were Mueller Hinton Agar (MHA) media (HIMDIA, India) and Potato Dextrose Agar (PDA), respectively. After the MHA and PDA media were prepared, they were incubated for 24 hours and then inspected for contamination. The sterile cotton bar was used to inoculate the test organism on media following a 24-hour incubation period. A pre-inoculated agar plate was gently filled with the sample disc for the 48-hour aerobic incubation of antifungal at 26 °C and antibacterial at 37 °C. Dimethyl sulfoxide (DMSO) was used as a control, and each disc was loaded with 25 μL of sample solution containing 300 μg of produced silver nanoparticles using Lantana camara leaf extract. For the antibacterial and antifungal assays, the same volume of Ceftriaxone and Amphotericin solution in DMSO was loaded per disc as the positive control, respectively. Following a 24-hour incubation period, the inhibition zone's circumference around the disc was measured in milli meters. Every experiment was carried out three times. In this investigation, two gram-positive strains of S. Aureus, B. Megnaterium, two gram-negative strains of S. typhi, E. coli, and two fungal strains of T. Harizianum and A. nigar were employed.

Chapter 4: RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF SILVER NANOPARTICLES

4.1.1 Physical Change

The starting stage of silver nanoparticle (AgNP) synthesis was identified by the color change of the reaction media in the aqueous solutions of silver nitrate (AgNO3) and *Lantana camera* leaf extract. AgNPs' surface plasmon excitation is the source of the color shift. As the reaction progressed, the color gradually changed, guaranteeing the conversion of Ag+ ions into Ag and the creation of silver nanoparticles. The reaction's color shift over time is depicted in Figure 4.1.

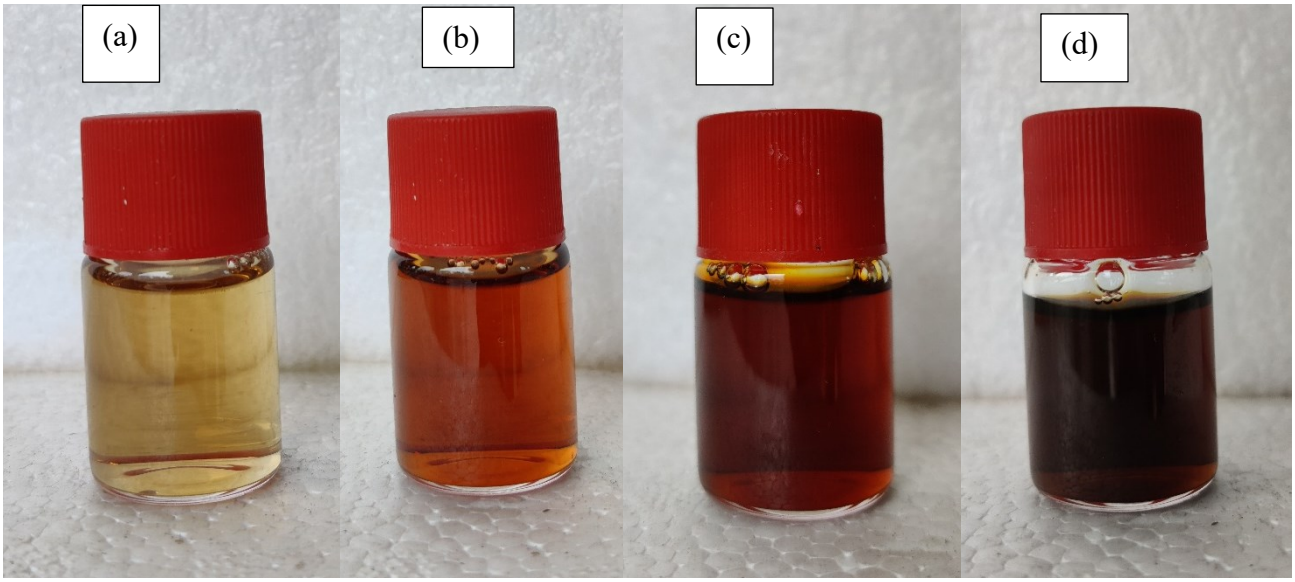


Figure 4.1 Changes of color with the formation of AgNPs (a) at the very initial time (b) after 30 minutes (c) after 1 hr (d) after 2 hr.

4.1.2 UV-Visible Spectroscopy Analysis

Preliminary characterization of biosynthesized AgNPs was confirmed by the UV-Visible spectroscopy. UV-Visible spectra of a mixture of plant extract and $AgNO_3$ at the very initial time and after the reaction of 2 hrs have been shown in Figure 4.2. At the onset of the reaction, no plasmon band was observed. The absorption band characteristic of spherical AgNPs is observed at 458 nm. There was no significant increase in absorption after 2 hrs which indicated the completion of the reduction and synthesis of AgNPs (Balavijayalakshmi & Ramalakshmi, 2017).

The visible range of AgNPs depends on their plasmonic properties. The plasmon of AgNPs responded in the range between 400-500 nm (Amendola et al., 2010). Free electrons of metal ions give the surface plasmon resonance absorption band (Jana et al., 2016). Only a single SPR band represented the spherical nanoparticles (Jana et al., 2016).

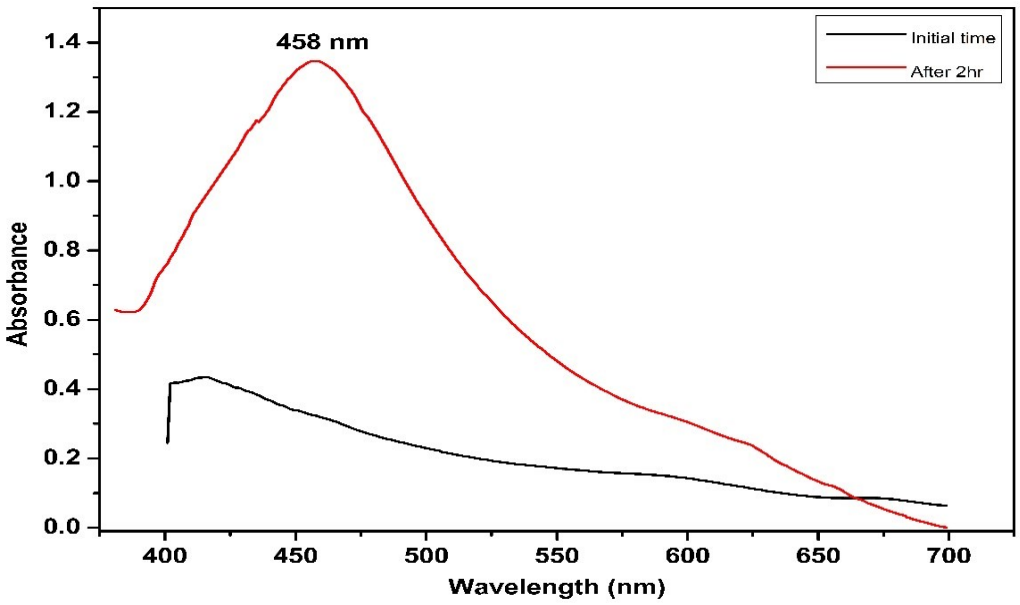


Figure 4.2 UV-Visible spectra of AgNPs at initial time and after synthesis.

4.1.3 Fourier Transform-Infrared (FT-IR) Spectroscopy Analysis

FT-IR analysis was employed to elucidate the functional groups responsible for the reduction of Ag from Ag^+ those present in the leaf of plant *Lantana camara*. Figure 4.3 shows there are four absorption peaks at wavenumbers 1615 cm^{-1}, 1510 cm^{-1}, 1209 cm^{-1}, 958 cm^{-1} corresponding to C=O stretching vibration of carbonyl groups, $-NO_2$ aliphatic nitro group, C-O stretching of the carboxylic group and unsaturated C=C bond, respectively which indicate a shift in absorption bands of the phytochemical compounds from aqueous extract of *Lantana camera* as a stabilizing agent (Adebayo-Tayo et al., 2019; Ajitha et al., 2014b; Khalil et al., 2014).

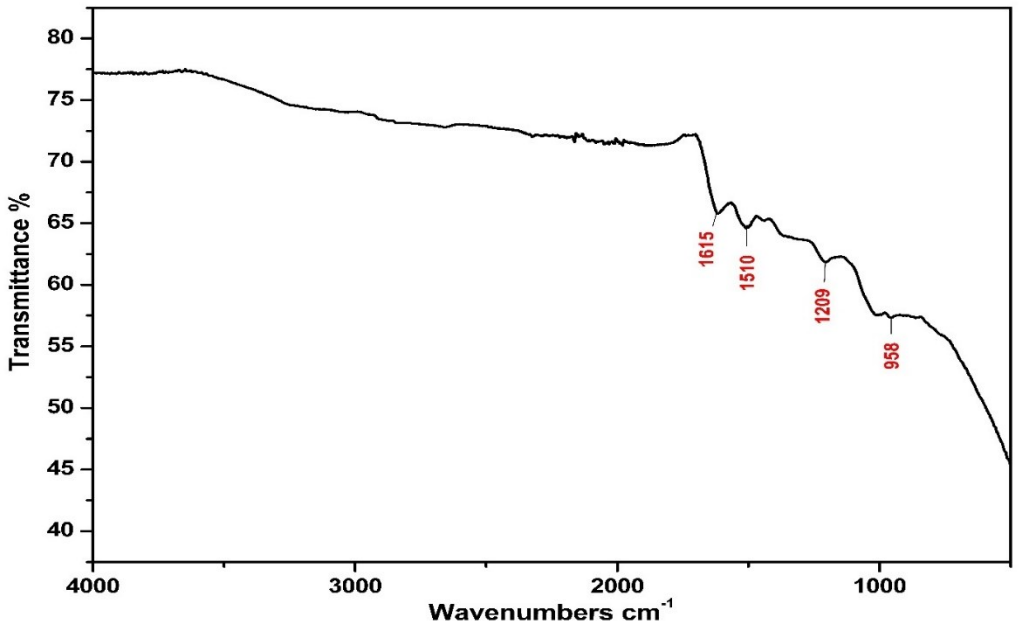
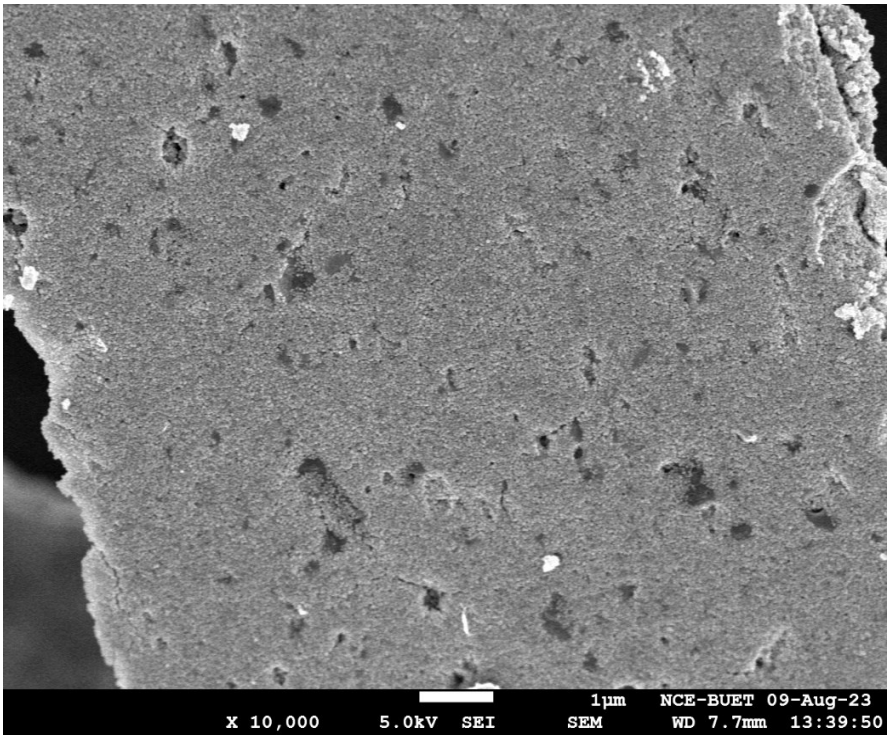


Figure 4.3 FT-IR spectrum of synthesized AgNPs.

4.1.4 Field Emission-Scanning Electron Microscopy (FE-SEM) Analysis

At different magnifications, SEM analysis was conducted using Field Emission-Scanning Electron Microscopy to evaluate the surface morphology of plant-mediated produced AgNPs. It displays some nanoparticle morphological characteristics, such as size, shape, and environment. Without any nanoparticle aggregation, spherical nanoparticles in the size range of 15 to 35 nm were observed. (Figure 4.4 and Figure 4.5).

(a)



(b)

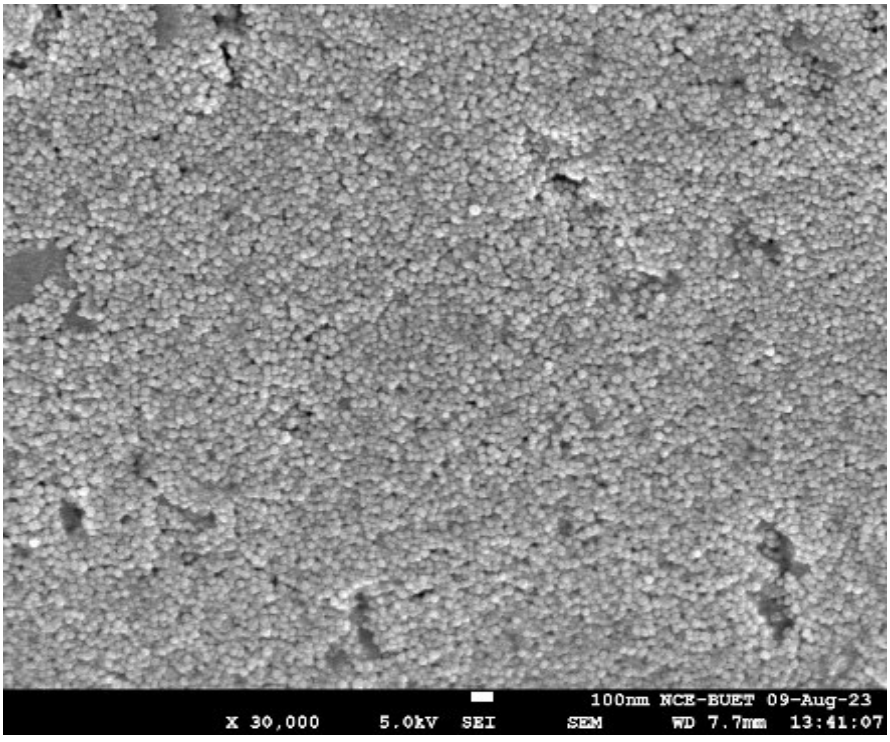
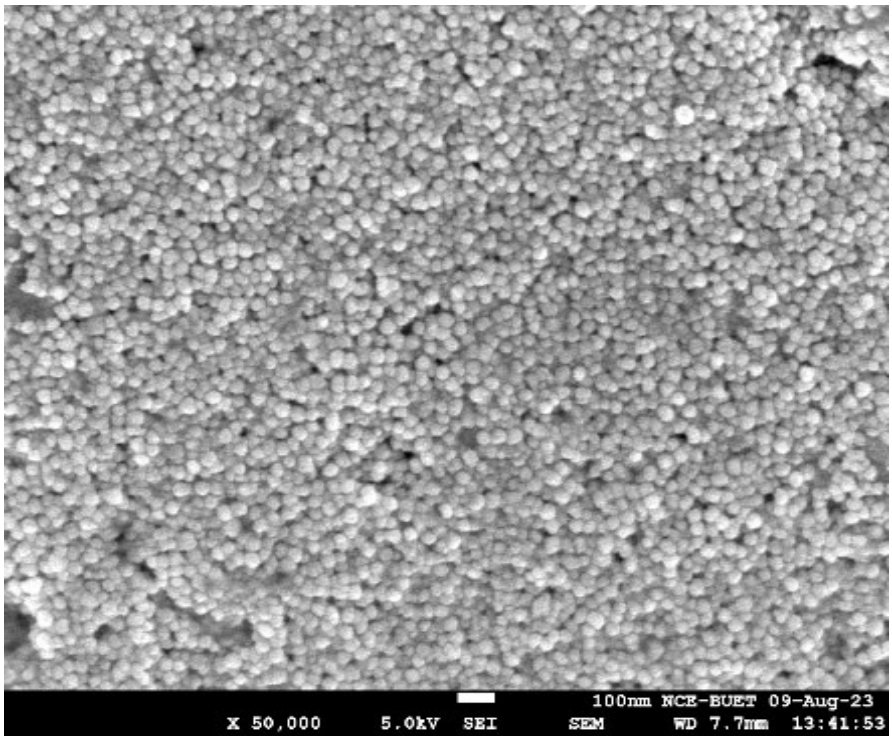


Figure 4.4 FE-SEM image of AgNPs at (a) 10,000 and (b) 30,000 magnifications

(a)



(b)

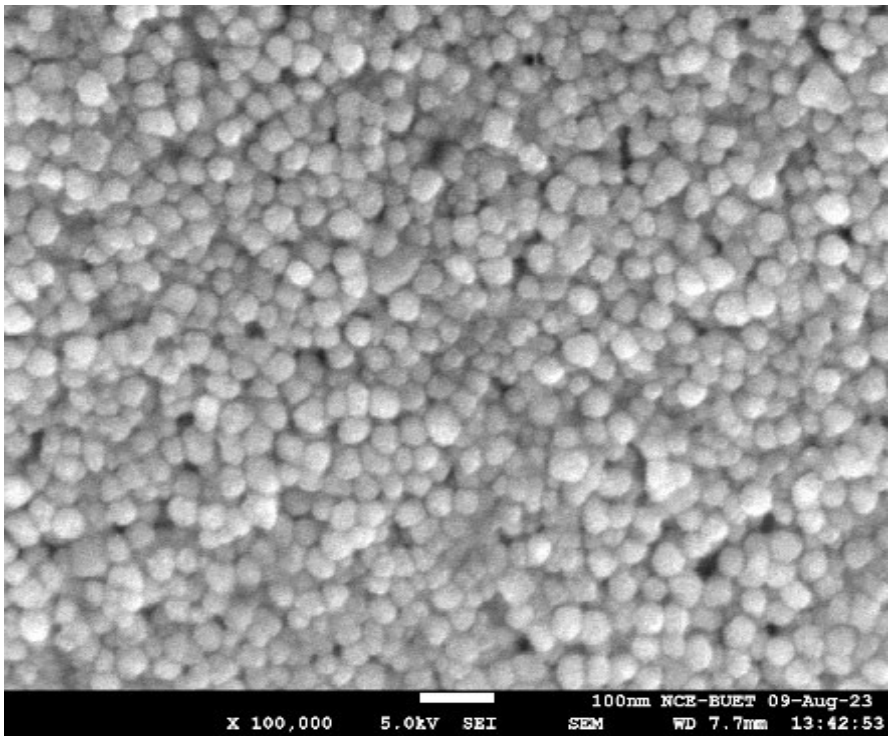


Figure 4.5 **a)** FE-SEM image of AgNPs at (a) 50,000 and (b) 1,00,000 magnifications.

4.1.5 Energy Dispersive x-ray (EDX) Analysis

Silver nanoparticle elemental analysis was performed using EDX spectroscopy. AgNPs production was confirmed by the high signal seen in the silver (Ag) region of the EDX spectra (Adebayo-Tayo et al., 2019). Metallic silver nanocrystals typically exhibit an optical absorption peak at about 3 keV due to the existence of the SPR band. The EDX study provides details on how the aqueous leaf extract of *Lantana camara* forms pure AgNPs. (Figure 4.6).

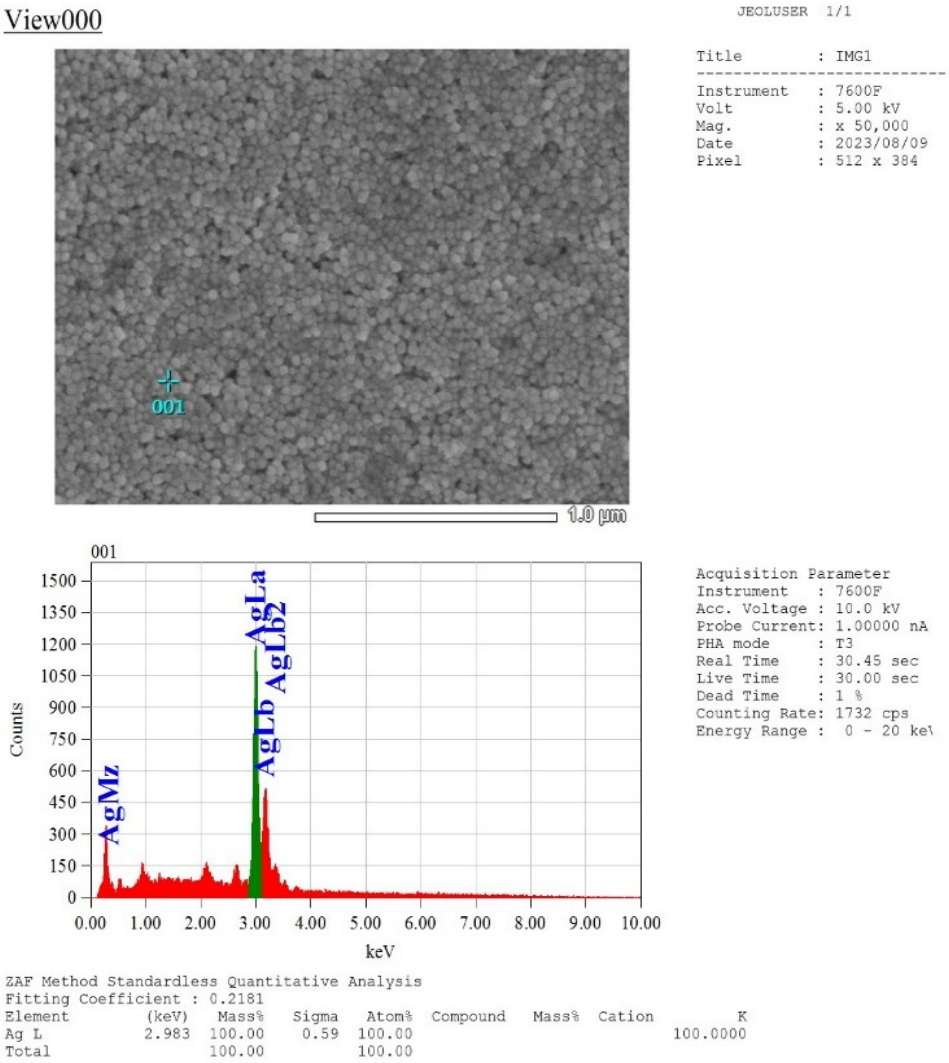


Figure 4.6 Elemental composition of AgNPs through EDX

4.1.6 X-Ray Diffraction (XRD) Analysis

The X-ray diffraction (XRD) peak pattern of the synthesized AgNPs by the leaf extract of *Lantana camara* is shown in Figure 4.7. The angle of diffraction data was taken for a range 30≤2θ≤80 degrees with a step of 0.01313 degrees. There are four peaks in the diffractogram at 38.15991, 44.10789, 64.54581 and 77.47817, these are the main characteristic peaks for AgNPs. The four distinct diffraction peaks at 38.16°, 44.10°, 64.56° and 77.48° of the 2θ values can be allotted to the planes of 111, 200, 220 and 300 respectively. By using Debye-Scherrer formula crystalline size and average crystallite size have been estimated from the diffractogram synthesized of AgNPs. Each one of the four peaks was fitted with a Gaussian function to find out the FWHM for the calculation of crystallite size. The FWHM of the fitted gaussian non-linear curve is taken as FWHM of the peaks in the diffractogram. These values are estimated through the software OriginPro 85. From the data of the four peaks "D" has been calculated. The average crystalline size of the synthesized AgNPs is found to be around 10 nm.

Calculation of crystallites size and average crystallites size from XRD data using Scherrer equation-

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Here, D = crustallite size (nm)

K = 0.9 (Scherrer constant)

β = Full Width at Half Maximum (FWHM) in radians

λ = 0.15406 nm (wavelength of X-ray sources)

θ = Peak position (radians)

Table 4.1. Coefficient for calculation of crystallinity of AgNPs

Peak No	2θ on x-axis	FWHM (deg)	Crystalline size 'D' (nm)	Average crystalline size (nm)
1	38.15991	0.86833	9.68	9.95
2	44.10789	2.13106	4.02	
3	64.54581	0.67211	13.98	
4	77.47817	0.83819	12.15	

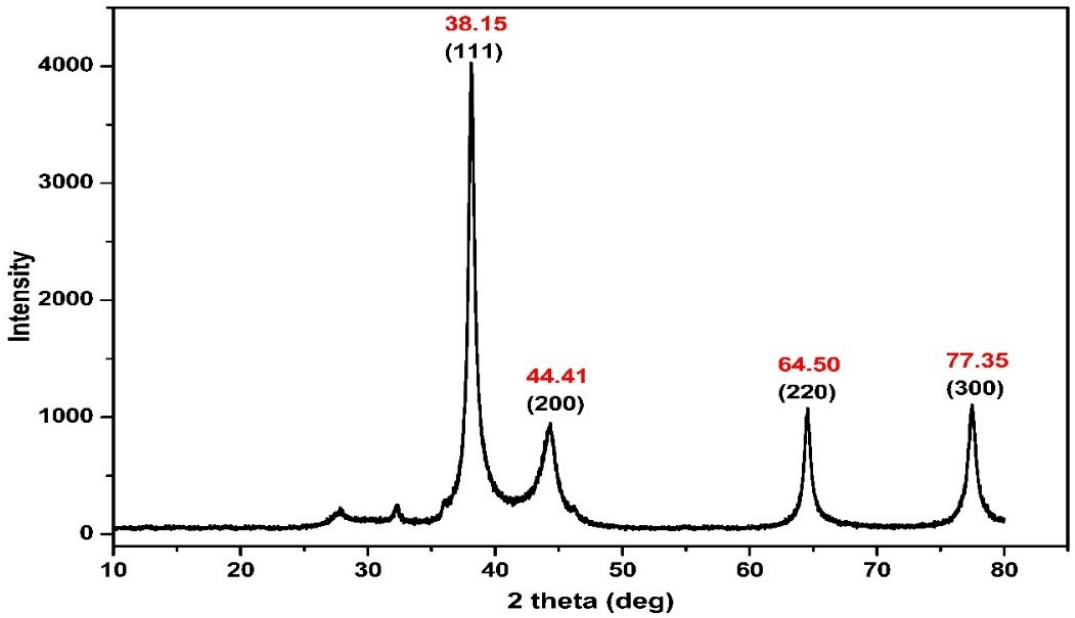


Figure 4.7 X-Ray Diffraction (XRD) peak pattern of synthesized AgNPs

4.2 PHOTOCATALYTIC ACTIVITY OF SILVER NANOPARTICLES

Photocatalytic activity of synthesized silver nanoparticles AgNPs were examined against cationic methylene blue dye under sunlight. The temperature and pH were in optimum condition as 25 °C and 7 respectively during the procedure. This photocatalytic activity examined against concentration of methylene blue dye 5 mgL^{-1} and 10 mgL^{-1}. The amount of synthesized AgNPs catalyst used for the degradation of methylene blue in each investigation was 125 mg, 250 mg and 500 mg for per litter of methylene blue solution. During the experiment absorbance of methylene blue dye was observed at 665 nm at different time intervals 0 min, 30 min, 60 min, 90 min and 120 min. For the 125 mg, 250 mg and 500 mg of catalyst against 5 mgL^{-1} of methylene blue solution the percentage of degradation was 95.05%, 95.77% and 95.55% respectively (Figure 4.8, Figure 4.9, Figure 4.10). The degradation of methylene blue dye is observed by measuring the absorbance at 665 nm, which is gradually decreased as the reaction continues until it reaches equilibrium after 120 min. Same procedure employed also for 10 mgL^{-1} of methylene blue dye solution. For 10 mgL^{-1} solution the degradation efficiency is around 50% with catalyst amounts 125 mg, 250 mg and 500 mg (Figure 4.11, Figure 4.12, Figure 4.13). Hence the photocatalytic activity of synthesized silver nanoparticles exhibited better results against the 5 mgL^{-1} solution of methylene blue dye.

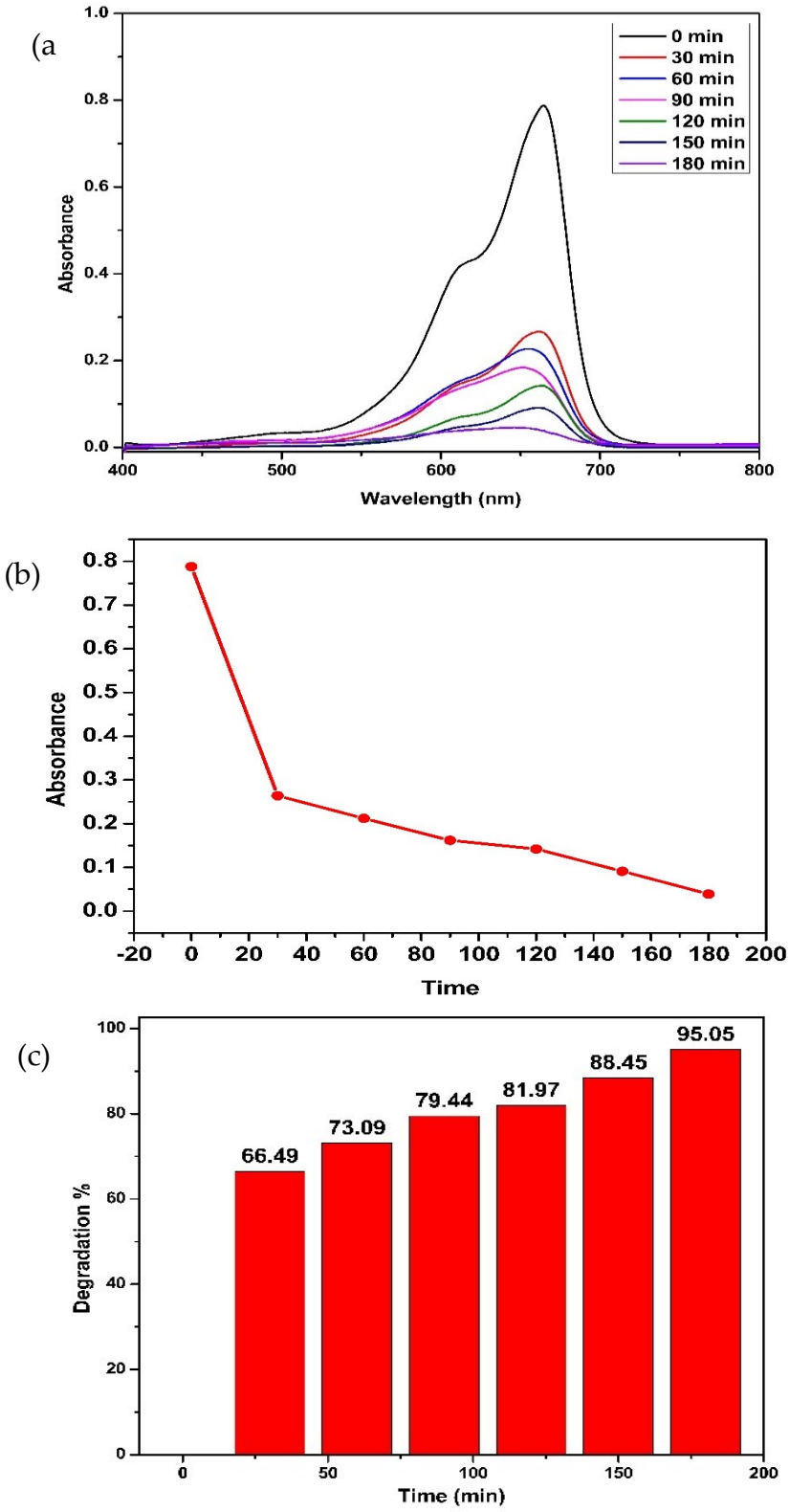


Figure 4.8 Photocatalytic degradation methylene blue solution (a) UV-visible spectra of degradation behavior along with time, (b) graphical representation of degradation of methylene blue with time, decreasing absorbance with time and (c) percent of degradation with time. Concentration of methylene blue = 5 mg L^{-1}, AgNPs catalyst = 125 mg.

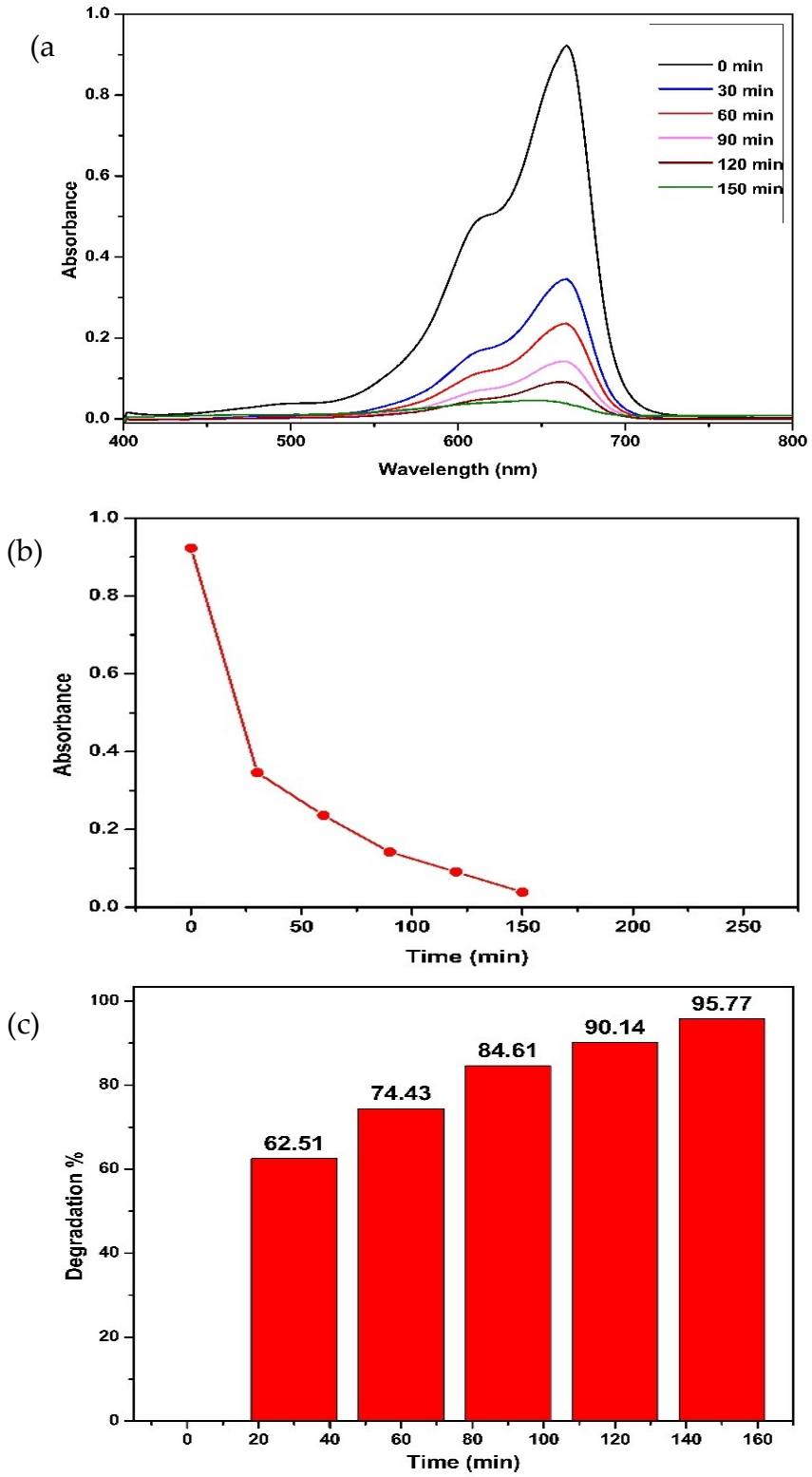


Figure 4.9 Photocatalytic degradation methylene blue solution (a) UV-visible spectra of degradation behavior along with time, (b) graphical representation of degradation of methylene blue with time, decreasing absorbance with time and (c) percent of degradation with time. Concentration of methylene blue = 5 mg L^{-1}, AgNPs catalyst = 250 mg.

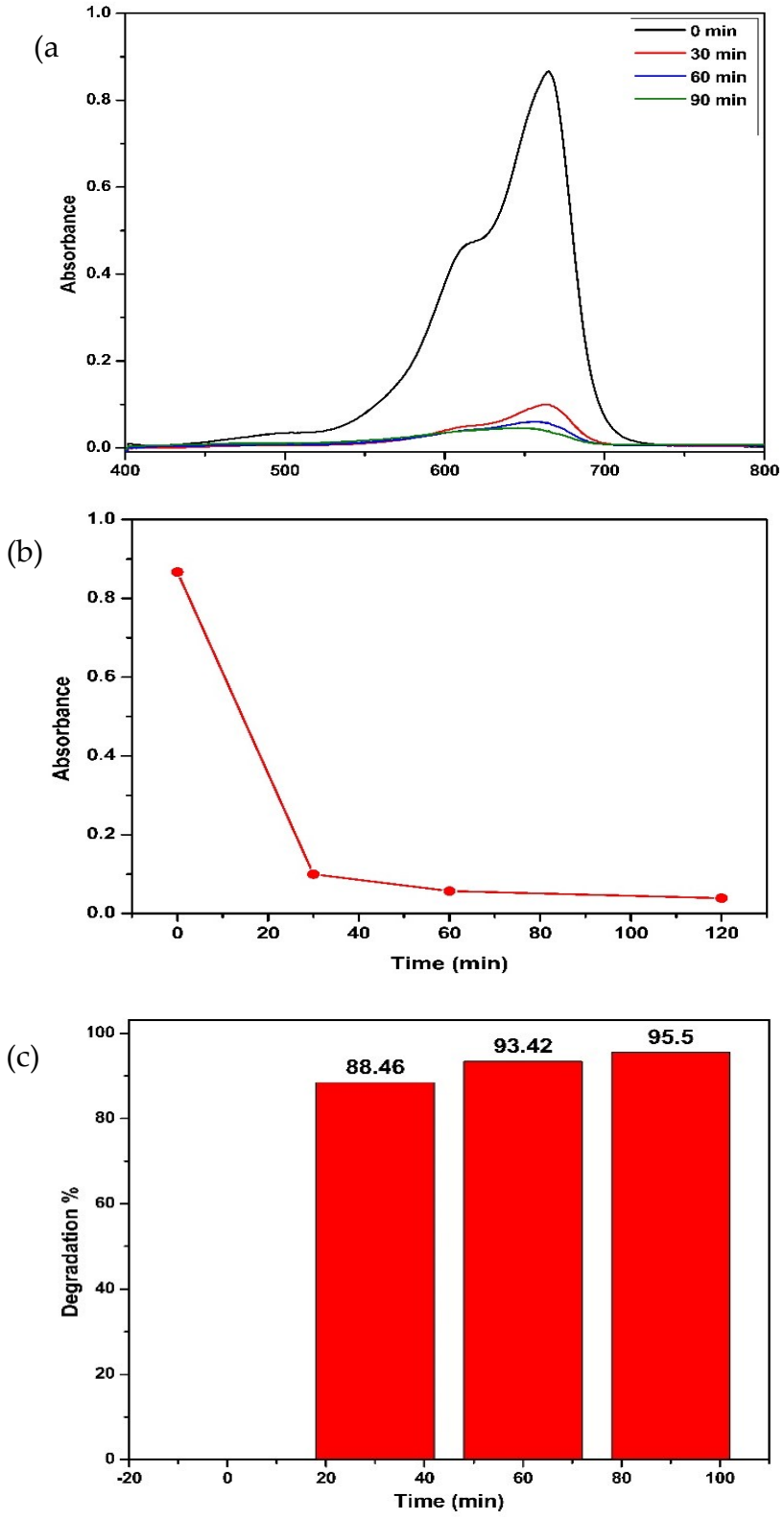


Figure 4.10 Photocatalytic degradation methylene blue solution (a) UV-visible spectra of degradation behavior along with time, (b) graphical representation of degradation of methylene blue with time, decreasing absorbance with time and (c) percent of degradation with time. Concentration of methylene blue = 5 mg L^{-1}, AgNPs catalyst = 500 mg.

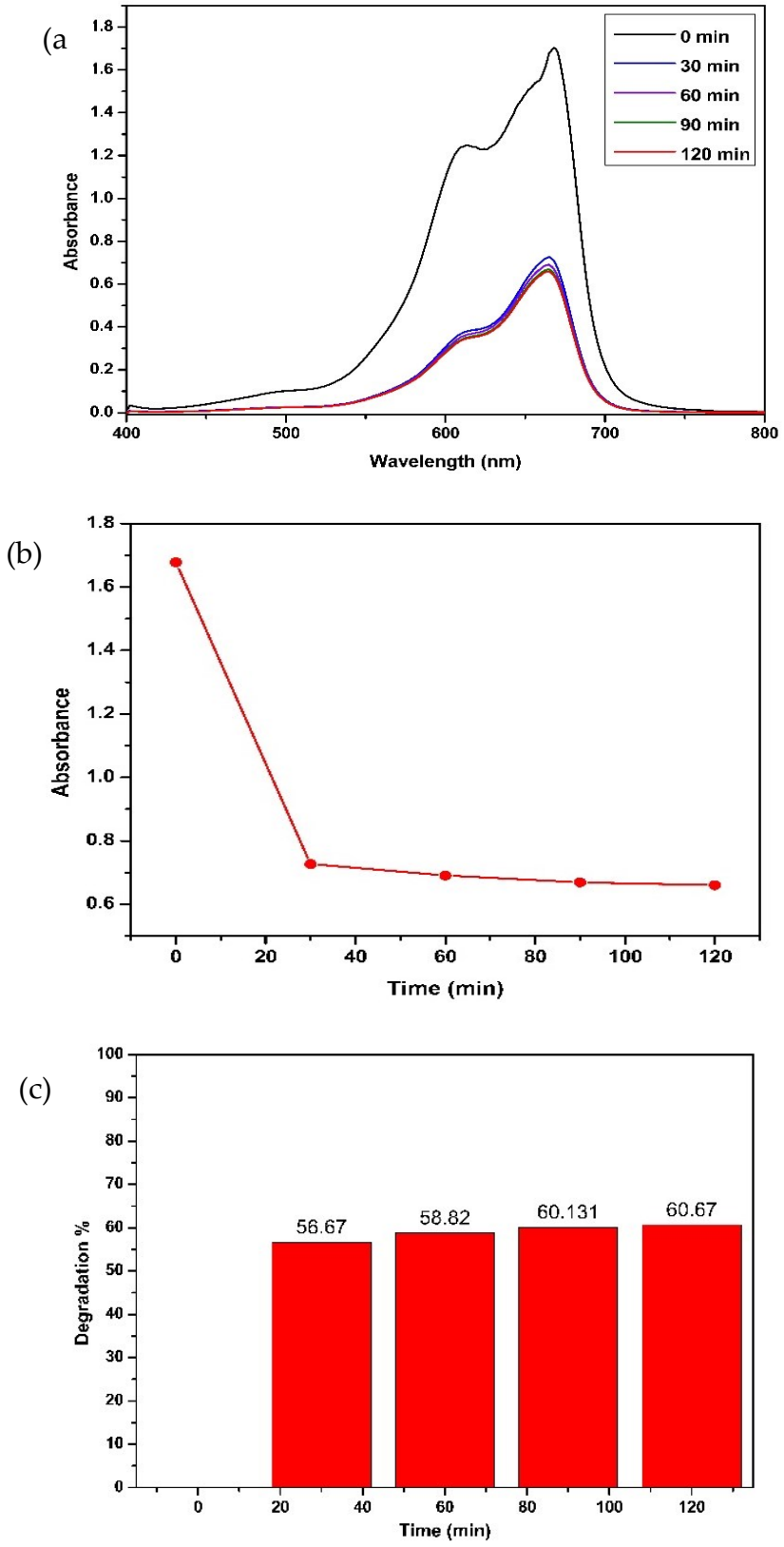


Figure 4.11 Photocatalytic degradation methylene blue solution (a) UV-visible spectra of degradation behavior along with time, (b) graphical representation of degradation of methylene blue with time, decreasing absorbance with time and (c) percent of degradation with time. Concentration of methylene blue = 10 mg L^{-1}, AgNPs catalyst = 125 mg.

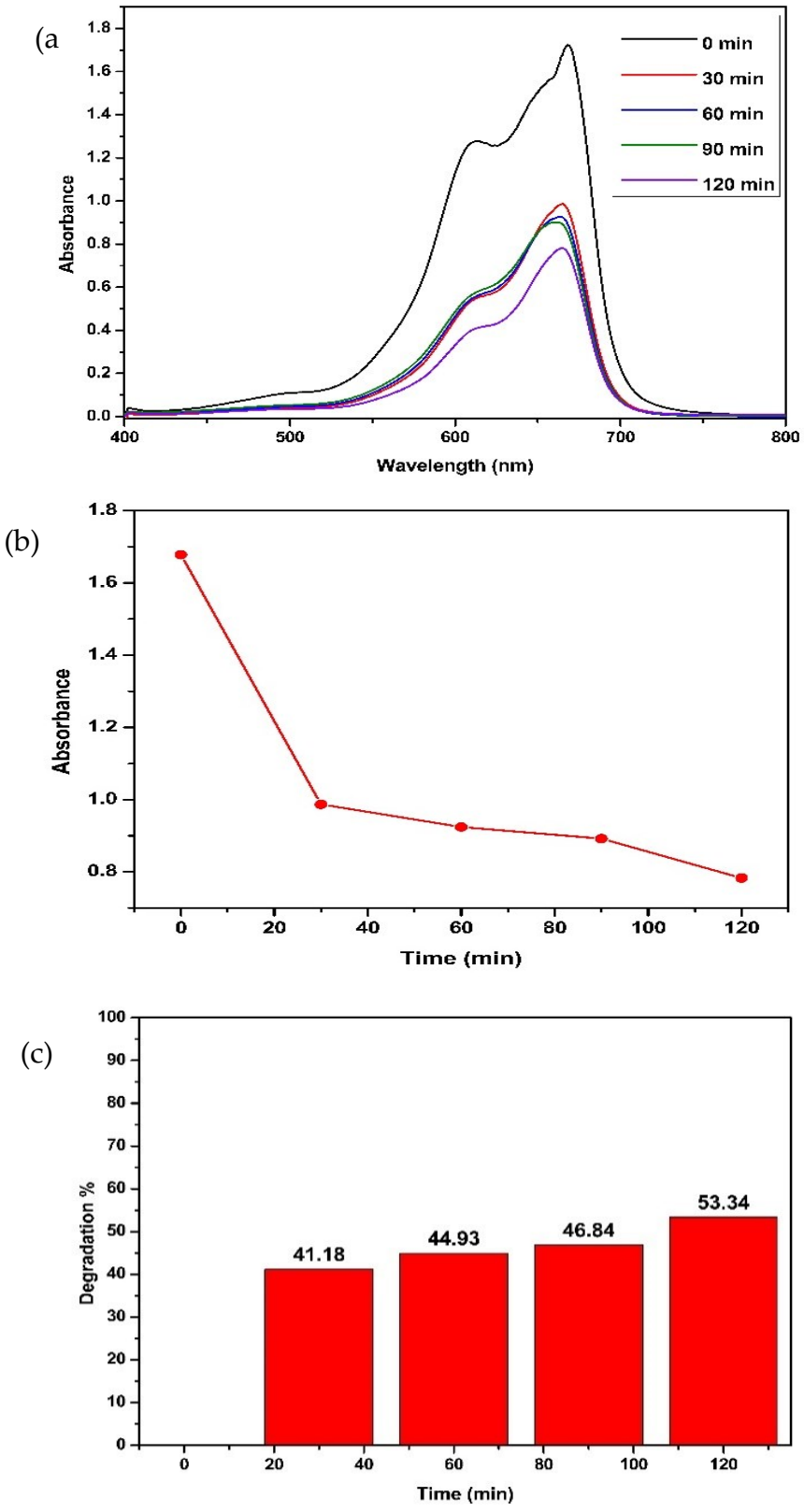


Figure 4.12 Photocatalytic degradation methylene blue solution (a) UV-visible spectra of degradation behavior along with time, (b) graphical representation of degradation of methylene blue with time, decreasing absorbance with time and (c) percent of degradation with time. Concentration of methylene blue = 10 mg L^{-1}, AgNPs catalyst = 250 mg.

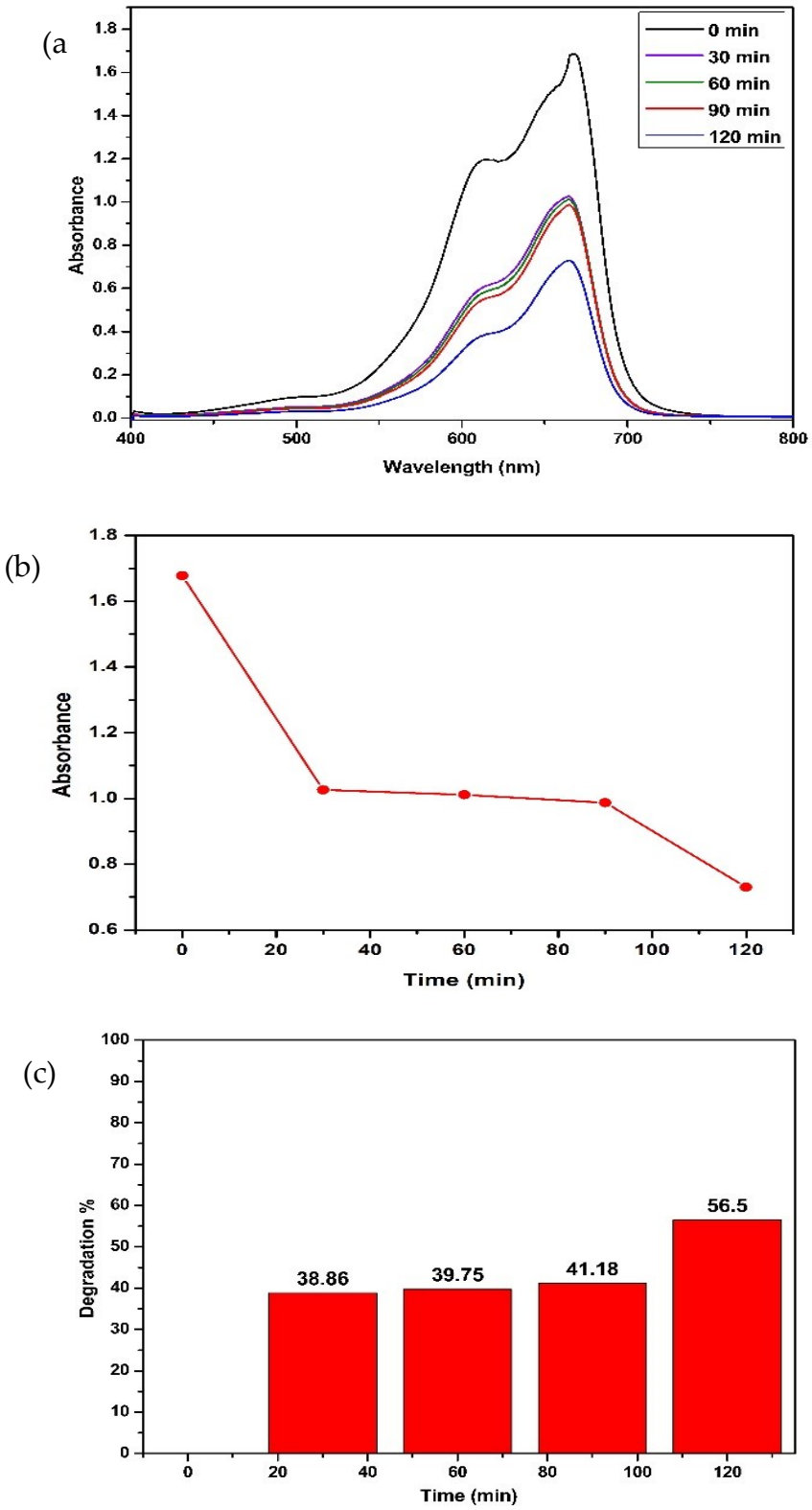


Figure 4.13 Photocatalytic degradation methylene blue solution (a) UV-visible spectra of degradation behavior along with time, (b) graphical representation of degradation of methylene blue with time, decreasing absorbance with time and (c) percent of degradation with time. Concentration of methylene blue = 10 mg L^{-1}, AgNPs catalyst = 500 mg

4.3 ISOTHERM STUDIES

To explicate the mechanism behind the degradation of methylene blue dye by synthesized AgNPs by leaf extract of *Lantana camara*, isotherm studies are essential (Chacón et al., 2006; Kansal et al., 2009; Konstantinou & Albanis, 2004; Nagaveni et al., 2004; Tanaka et al., 2000; Tomar et al., 2020). Approaching the equilibrium phase of degradation by isotherm studies the investigation was performed at different four initial concentrations of methylene blue dye at room temperature with constant pH and exposure time of 0 to 120 min. Langmuir linear isotherm (Equation 1, Figure 4.14(a)) and Freundlich linear isotherm (Equation 2, Figure 4.14(b)) models were used to examine the maximum degradation amount of MB dye.

$$\frac{C_e}{q_e} = \frac{1}{K_L \times q_m} + \frac{C_e}{q_m} \qquad (1)$$

where, C_e = initial concentration of methylene blue dye

q_e = degradation quantity

q_m = maximum degradation capacity

K_L = Langmuir constant

$$ln\ q_e = ln\ K_f + \frac{1}{n} ln\ C_e \qquad (2)$$

where, C_o = concentration of dye at initial time

C_e = concentration at time t,

K_f = Freundlich constant

Table 4.2. Coefficient of isotherm models for degradation of methylene blue

Material	**Langmuir isotherm model**		
	K_L	**q_m**	**R^2**
AgNPs	0.040378	24.76607	0.883593

Material	**Freundlich isotherm model**		
	K_F	**n**	**R^2**
AgNPs	8.718381	1.247538	0.990837

Based on the obtained data presented in above table, this experimental data was better fit to Freundlich isotherm model with the correlation value of 0.990837.

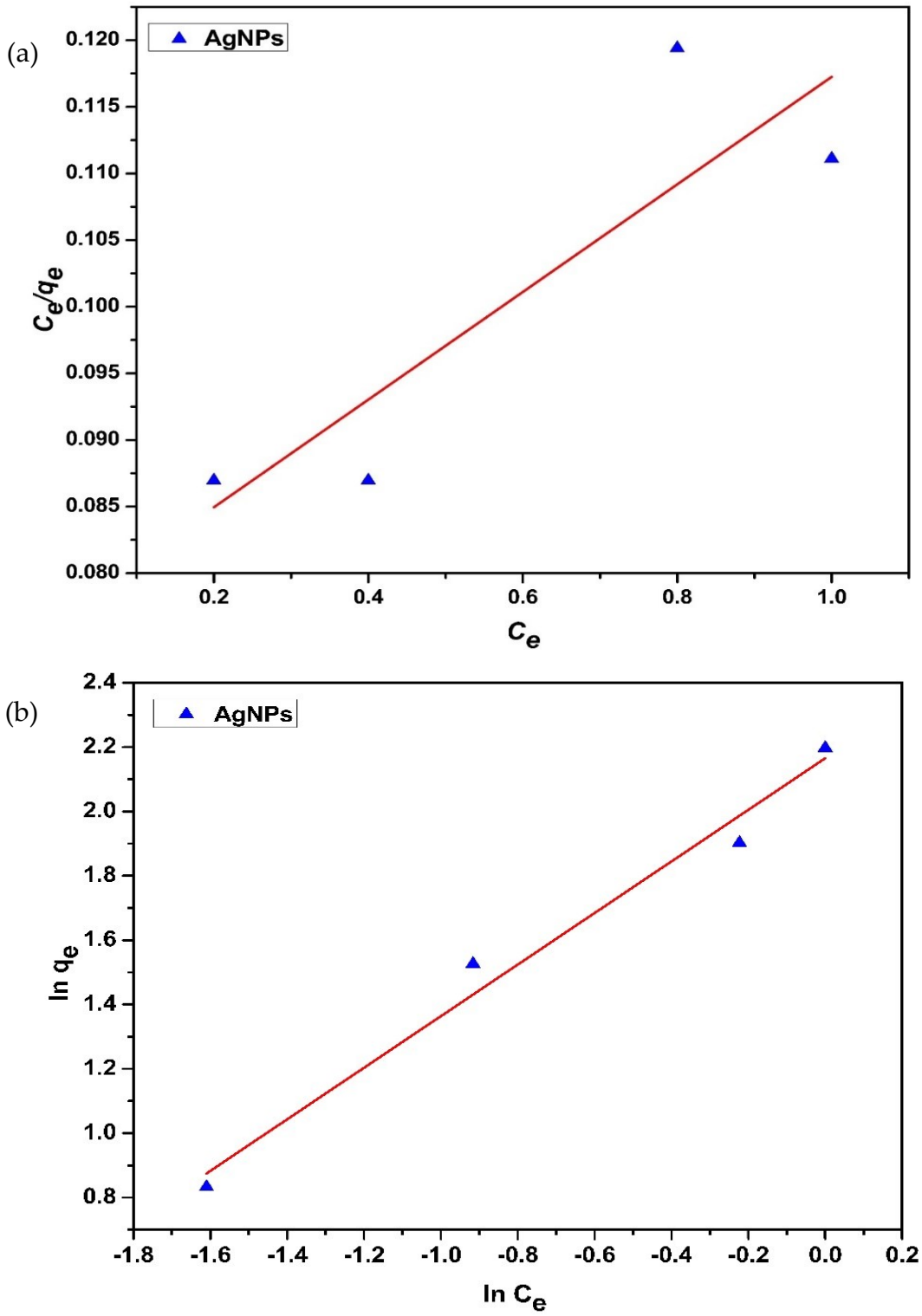


Figure 4.14 Isotherm model (a) Linear plots of the Langmuir isotherm and (b) Linear plots of Freundlich isotherm. Conditions: concentration of MB dye 2.5 mgL^{-1}, 5 mgL^{-1}, 7.5 mgL^{-1}, 10 mgL^{-1}; catalyst 250 mg L^{-1}; pH 7; 25 °C

4.4 REACTION KINETICS STUDIES

Degradation rate of methylene blue by synthesized AgNPs was investigated through kinetics study (Figure 4.15)(Chacón et al., 2006; Mahmoodi et al., 2006; Molinari et al., 2004; Rauf & Ashraf, 2009; Sauer et al., 2002). To evaluate the kinetics of degradation of methylene blue dye by synthesized AgNPs, the experiments were carried out for a concentration of 10 mgL^{-1} of methylene blue at room temperature 25 °C, the amount of catalyst being applied for the procedure was 250 mg per litter of methylene blue solution. The experiment was carried out for 0 min to 120 min when the reaction reached the equilibrium. The kinetics of degradation of methylene blue was investigated with a pseudo-first-order (Equations 3 and 4, Figure 4.16a) and pseudo-second-order model (Equations 5 and 6, Figure 4.16b).

Pseudo-first-order Eq.

$$q_t = q_e\,(1 - e^{-kt}) \qquad (3)$$

linear form of pseudo-first-order Eq.

$$ln(q_e - q_t) = ln\ q_e - kt) \qquad (4)$$

Pseudo-second-order Eq.

$$q_t = kq_e^2 t/(1 + kq_e t) \qquad (5)$$

linear form of pseudo-second-order Eq.

$$t/q_t = 1/kq_t^2 + t/q_e \qquad (6)$$

where q_e and q_t are the concentration of methylene blue at equilibrium and time (t), respectively, also the co-efficient of each model is listed in the Table

The linearity in the pseudo first-order plots is better than that of pseudo second-order plots shown in the Figure 4.16, as confirmed by the higher R^2 values. This pseudo first-order kinetic model exhibits the chemical reaction of the experiment and its rate of removal of methylene blue dye.

Table 4.3. Coefficient of kinetics models

Material	**Pseudo first-order model**	
	K	**R^2**
AgNPs	0.018632	0.99237

Material	**Pseudo second-order model**	
	K	**R^2**
AgNPs	0.001963	0.952119

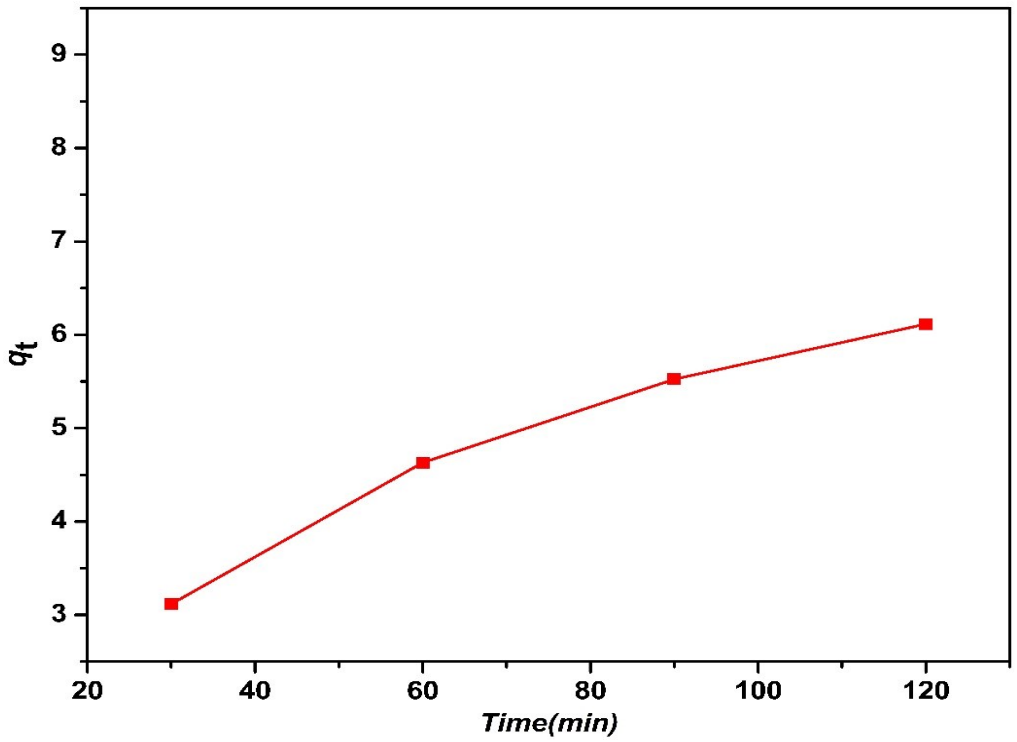


Figure 4.15 Amount of degradation of methylene blue with time

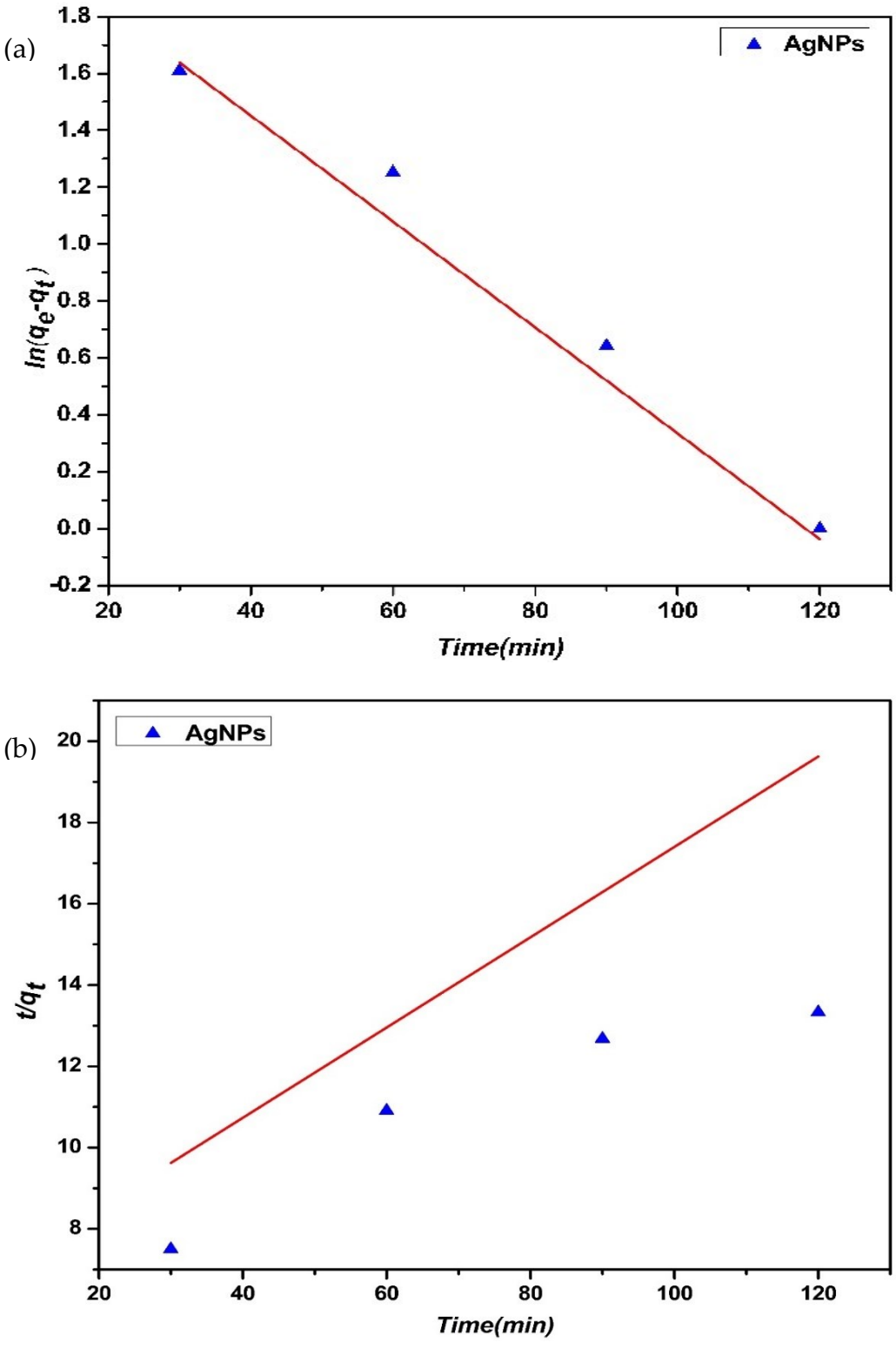
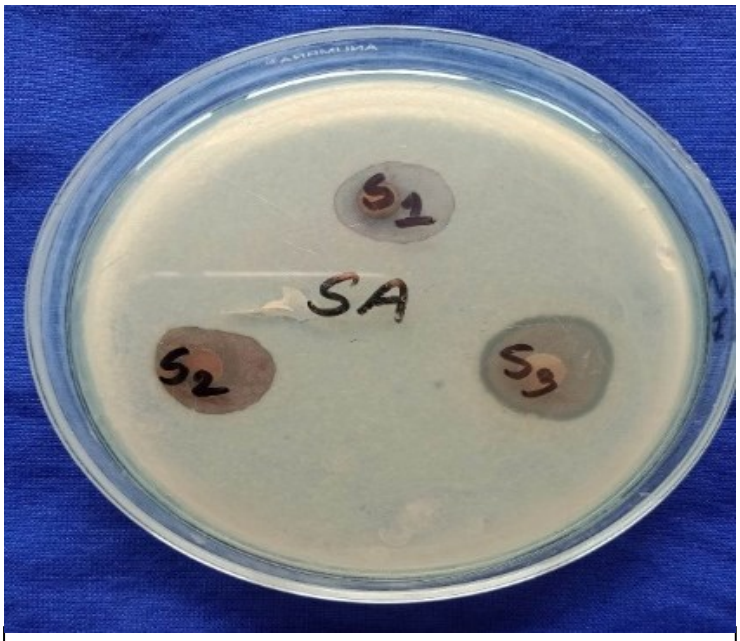


Figure 4.16 Linear plots of (a) pseudo first-order kinetics and (b) pseudo second-order kinetics of degradation of methylene blue dye by AgNPs. Initial concentration of MB dye 10 mgL^{-1}; catalyst 250 mgL^{-1}; pH 7; 25 °C

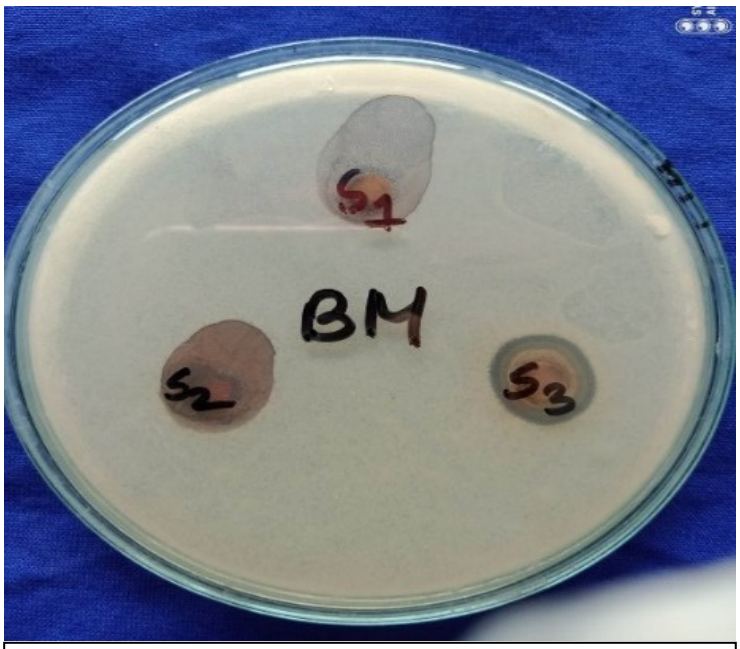
4.5 MICROBIAL ACTIVITY ASSAY

Utilizing the agar disc diffusion method to assess the inhibition zone, synthesized silver nanoparticles derived from *Lantana camara* leaf extract were used to investigate the in vitro antimicrobial activities against two gram-positive strains (S. aures and B. megnaterium) (Figure 4.17), two gram-negative strains (S. typhi and E. coli) (Figure 4.18), and two fungal strains (T. harzianum and A. niger) (Figure 4.19). Table 4.4 displays the inhibitory zone execution measured in diameter (mm ± SD). Ceftriaxone and Amphotericin B. were used as standard. The samples were used to evaluate the antimicrobial study S1, S2 and S3 were in different pH e.g. 7, 3, and 12 respectively. S1 and S2 showed activity against all of the microbes gram-positive, gram-negative and fungal strains where S2 which was in pH 3 didn't show activity against gram-positive and gram-negative but showed better activity against two fungal strains TH and AN compared to standard as their inhibition value 20±0.6 and 19.5±0.5 respectively. All samples showed better activity against fungal strains compared to standards. Activities of S1 and S2 were below the inhibition value of standard for gram-positive and gram-negative bacteria shown in the Table 4.4.



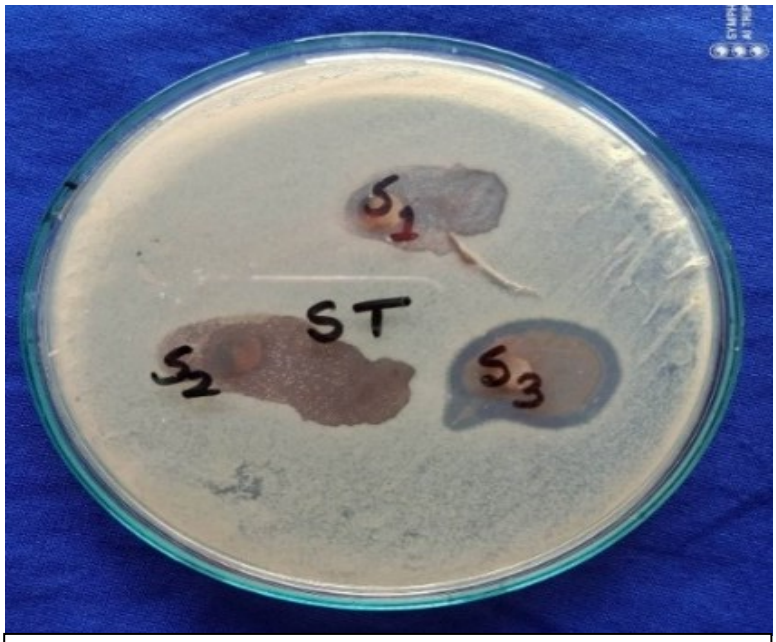
S. aures

Gram (+) Positive Bacteria



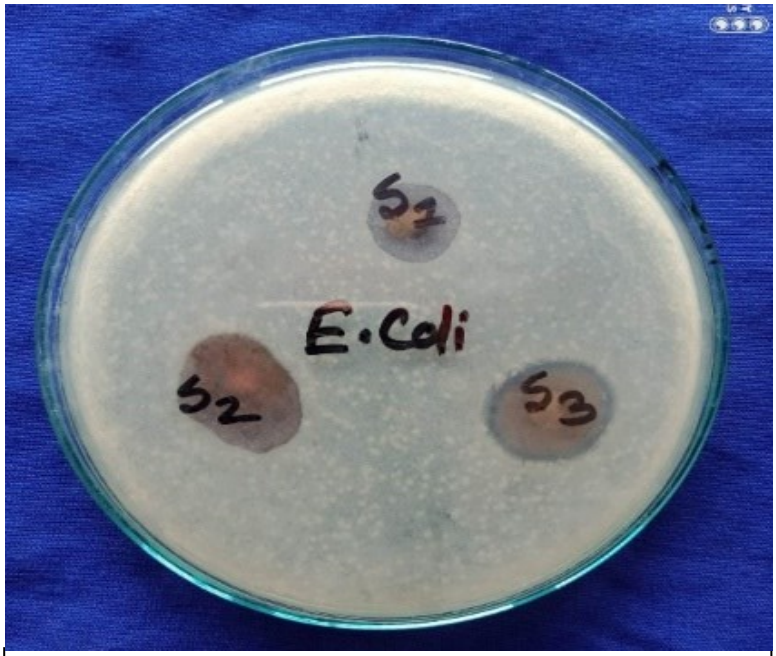
B. megnaterium

Figure 4.17 Antimicrobial activity, Inhibition zone against Gram-positive bacteria



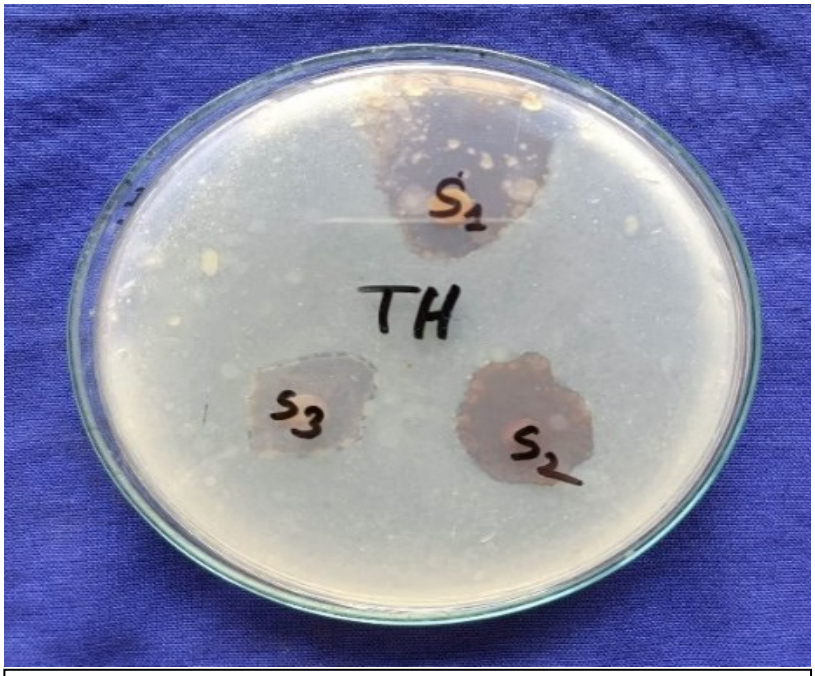
S. typhi

Gram (-) negative Bacteria



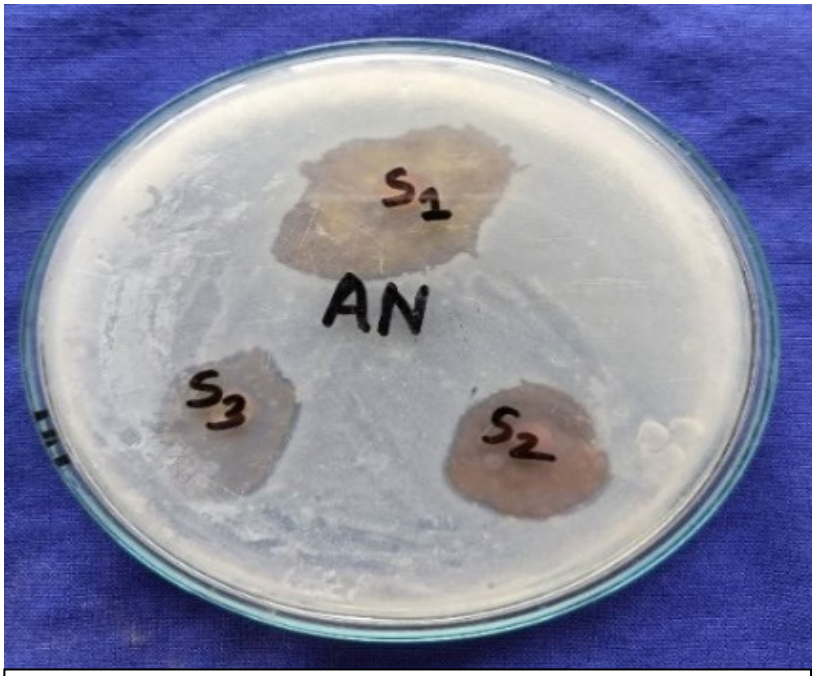
E. coli

Figure 4.18 Antimicrobial activity, Inhibition zone against Gram-negative bacteria



T. harzianum

Fungal strains



A. niger

Figure 4.19 Antimicrobial activity, inhibition zone against fungal strains

Table 4.4. Determination of inhibition zone (mm ± SD) of the synthesized AgNPs with *Lantana camara* aqueous leaf extract, Ceftriaxone and Amphotericin B. against gram-positive, gram-negative bacteria and fungal strains.

Sample	**Gram-positive**		**Gram-negative**		**Fungi**	
	SA	**BM**	**ST**	**EC**	**TH**	**AN**
S1	13.5±1.0	17±0.5	17±0.6	12±1.0	29±1.0	23±1.0
S2	-	-	-	-	20±0.6	19.5±0.5
S3	16±1.0	12±1.0	21±1.0	17±0.5	16±1.0	19.5±1.0
Ceftriaxone	38.0±1.0	34.0±1.0	44.3±0.6	40.0±1.0	-	-
Amphotericin B	-	-	-	-	17.7±0.5	8.3±0.5
DMSO	-	-	-	-	-	-

[1]The data are mean ± SD (standard deviation) (n = 3). – Represents no activity.

Chapter 5: CONCLUSION AND RECOMMENDATION

5.1 CONCLUSIONS

The outcome of this present work indicates the synthesis of silver nanoparticles (AgNPs) with *Lantana camara* aqueous leaf extract successfully accomplished. Synthesis of silver nanoparticles using this plant leaf extract is an eco-friendly process toward green approaches of nanoparticle synthesis. This synthesis process ensures that it is free from any kind of toxic and hazardous chemical that is being used in any other conventional methods like chemical methods. Being a native and wild plant, the phytochemical constituents of *Lantana camara* could vary from region to region. Implying this point, the present work established that, the synthesis of silver nanoparticles was successfully done with this region's plant distributed at Chattogram in Bangladesh. Synthesis of AgNPs confirmed using UV-visible spectrophotometer observing Surface plasmon resonance band peak at 458 nm. Further characterization was investigated with FE-SEM, EDS and XRD analysis. Field Emission-Scanning Electron microscopic analysis ensured the spherical shape and surface morphology with unimolecular distribution. The average size of synthesized AgNPs is approximately 30 nm. Energy dispersive X-ray analysis assured purity of AgNPs with 100% mass which do not contain any kind of impurity and also exhibit characteristic peaks at 3 keV for AgNPs. Crystalline nature and size calculated from XRD data is 9.8 nm. All of these analyses confirmed the successful synthesis of AgNPs from *Lantana camara* aqueous leaf extract.

Considering various applications of silver nanoparticles in different sectors, synthesized silver nanoparticles in this present work also exhibit some

characteristics as application. Synthesized silver nanoparticles showed good catalytic activity as photocatalytic activity against synthetic organic dye Methylene Blue. Photocatalytic activity was also examined in an environment friendly way which excluded any kind of harmful radiation. This activity was investigated in criteria that is suitable for lab experiments and it can be employed in a broad and wide area over various sectors. Synthesized AgNPs were employed for the degradation of methylene blue dye and exhibited a 95.77% degradation rate on a dosage of 250 mg per liter of methylene blue solution having a concentration of 5 mgL^{-1}. Other dosages 125 mg and 500 mg showed degradation rates 95.05% and 95.50% respectively.

Also *in vivo* activity of synthesized silver nanoparticles as one of the biological applications of silver nanoparticles being done in this present work. Synthesized silver nanoparticles showed moderate anti-microbial activity against four bacterial strains (gram positive and gram negative) and two fungal strains hence ensured that synthesized silver nanoparticles have biological activity against these microorganisms. Hence it can be applicable in a wide range including various biological applications. AgNPs showed very good activity against anti-fungal strains compared to standards with inhibition zone 29±1.0 ,23±1.0 for *T. harzianum and A. niger* respectively.

Isotherm and reaction kinetics studies elucidated for synthesized AgNPs as Langmuir, Freundlich isotherm model, and pseudo first-order, pseudo second-order model. Experimental data showed best fitting to Freundlich isotherm model with the correlation value R^2 0.990837 and showed best fitting to pseudo first-order with the correlation value R^2 0.99237.

5.2 RECOMMENDATIONS OF THIS RESEARCH

Water sources beneath the mother earth are getting polluted day by day which is asking a great attendant to get it out from this disaster as early as possible. There are many kinds of pollutants being discharged by living beings which makes the water sources vulnerable for human health. Among these harmful effluents dyes are the toxic and very harmful ones. So this is a big matter of concern regarding future aspects.

Nanoparticles can play a crucial role as catalysts for removing various dyes from the water body. Approaching green synthesis methods nanoparticles can be synthesized using various natural sources which are free from any kind of toxic and hazardous chemicals.

So green synthesis method of nanoparticles conquering some great advantages as:

- An environment friendly synthesis process
- Easy synthesis method
- Free from any kind of toxic and hazardous components
- Nanoparticles with well define size and morphology
- Energy consuming and cost effective

And nanoparticles can be employed to remove various organic effluents and dyes from water sources and water bodies approaching their enhanced catalytic activity against these different effluents.

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Appendices

Appendix A:

1) Photographs