

pH modulated green synthesis of silver nanoparticles of *Ziziphus mauritiana* for its anti-pollutant activity

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By

Ms. Priya Singla

Roll No.301507005

Under the Supervision of

Dr. Diptiman Choudhury

Assistant Professor



SCHOOL OF CHEMISTRY & BIOCHEMISTRY

THAPAR UNIVERSITY, PATIALA-147004

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CERTIFICATE

I hereby declare that the thesis entitled “**pH modulated green synthesis of silver nanoparticles *Ziziphus mauritiana* for its anti-pollutant activity**” being submitted by Ms. Priya Singla in partial fulfillment of requirement for the award of the degree of **Masters of Science** in Biochemistry from **School of Chemistry and Biochemistry, Thapar University, Patiala**, is a bonafied work carried out under the supervision of **Dr. Diptiman Choudhury** and no part of this project has been submitted for award of any degree by me.



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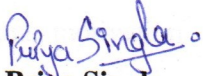
Assistant Professor (SCBC)

Thapar University, Patiala

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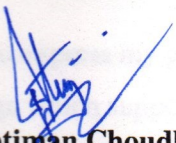
I hereby declare that the work being presented in the dissertation entitled "**pH modulated green synthesis of silver nanoparticles *Ziziphus mauritiana* for its anti-pollutant activity**" in the partial fulfillment of the requirements for the award of the degree of Masters in Biochemistry and being submitted to School of Chemistry and Biochemistry, Thapar University, Patiala, is my own work during the period of January to July 2017, under the supervision of **Dr. Diptiman Choudhury**. I have not submitted the contents embodied in this dissertation for the award of any other degree.

Patiala


Priya Singla

Date: 17-July-2017

This is to certify that the above statement made by the candidate is correct and true to the best of my knowledge.


Dr. Diptiman Choudhury

Project Supervisor

Assistant Professor (SCBC)

Thapar University, Patiala

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ABSTRACT

Environment is getting polluted due to frequent release of waste from the industries. Thus requirement of renewable sources is required that could help in reduction of various harmful products and act as eco- friendly catalysts. The advancement of nanotechnology can be utilized to enhance water quality. A few techniques that can utilize nanotechnology utilize receptive media for partition and filtration, bioremediation and sterilization. Remediation is the procedure to evacuate, limit or kill the water contaminants that can harm human wellbeing or biological communities.

The present work investigates the synthesis of silver nanoparticles (AgNPs) by biological method using *Ziziphus mauritiana*. Silver nanoparticles were successfully synthesized from *Ziziphus mauritiana* by green synthesis method at different pH range (pH 3, 5, 7, 9, 11, 13). This synthesis was confirmed by surface plasmon resonance which resulted as with the increase of pH range the size of the nanoparticles decreases. Particle extinction coefficient was monitored by the UV-visible spectroscopy. Morphological studies were done using DLS, Zeta Potential and FTIR. The catalytic activity of AgNPs was monitored by antibacterial activity against *E.coli* and *Bacillus subtilis* and other properties of catalytic degradation were confirmed by the adsorption of dyes and reduction of pesticides. Thus catalytic activities concluded that with the increase in the pH range the size of the AgNPs decreases and catalytic activity increases.

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ABBREVIATIONS AND SYMBOLS

Abbreviation	Name
°C	Degree Celsius
%	Percent
G	Grams
Mg	Milligram
Min	Minutes
Conc.	Concentration
Nm	Nanometer
FTIR	Fourier Transform Infrared Spectroscopy
HCl	Hydrochloric acid
Vs.	Versus
ZAE	<i>Ziziphus mauritiana</i> aqueous Extract
AgNPs	Silver nanoparticles
NPs	Nanoparticles

CHAPTER 1

INTRODUCTION

1.1 Water pollution and pollutants:

The unfavorable change caused in natural environment due to the presentation of contaminants is pollution. There are three noteworthy types of contamination which are specified underneath: Air contamination is characterized as the nearness of poisonous chemicals or mixes which bring down the nature of air or make hindering changes personal satisfaction, for example, causing a dangerous atmospheric deviation or harming of ozone layer (Danilov-Danil'yan et al. 2009). Water contamination happens when substances enter water bodies, getting broken up in them, lying suspended in water, this declines nature of water. Water contamination is caused by the release of wastewater from business and mechanical waste into surface waters; releases of inorganic toxins, for example, alkalis, acids, polyphosphate cleansers from substance ventures, release of poisonous metals, for example, Hg, Cd, Pb, Co, Mn and Cr release of pesticides like insecticides and herbicides. The vast majority of the water is put away in seas and ice tops, which is hard to be recovered. Around 98% of this water is seawater but since of the high grouping of salt, it is unusable for drinking. Aggregate sum of new water exhibit is just 2%, yet polar ice tops and ice sheets bolt up to 1.6% of it (Cheremisinoff 2001). Pure water will be water from a source that has evacuated all debasements. Water which has low disintegrated solids and upsetting gases and additionally low in organic life is thought to be immaculate water and henceforth utilized for various reasons as drinking. At the point when water gets changed in its quality or structure either actually or because of human exercises to wind up plainly less appropriate for drinking, household, modern, agrarian or different utilizations for which it would have been generally reasonable in its characteristic or unmodified state, can be viewed as contaminated water. Substances called poisons causes undesired impact on condition or harms the assets value. Contaminations like cleansers, oil hydrocarbons, herbicides and bug sprays, manures, smelling salts from sustenance preparing waste and colors causes water contamination. Soil contamination is unpredictable deforestation, burrowing for minerals, annihilation of touching area for human residence have done unsalvageable harm to the land and even prompted cruel climatic changes. Strong waste, frequently called the third contamination after air and water contamination, is that disposed of material which emerges from human exercises. The yearly strong waste creation is: household and exchange 8.5%, enterprises 15.2%, warm power stations 7.3%, mining 67% and development 2%.



(a)



(b)

Fig 1: (a) and (b) Release of dyes in water lead to water pollution

1.2 Methods of controlling water pollution:

Release volumes can be decreased by reduction of direct modern release volumes into the metropolitan sewer system and by reduction in infiltration into city sewer frameworks. The three general classifications, for control of pathogenic microscopic organisms, infections and parasites are – control at the input source of the micro-organism, municipal wastes are the principal inputs of communicable disease organisms and these inputs can be reduced by treatment of wastes without disinfection and disinfection by chlorination, ozonation, and ultraviolet radiation. Controls of the area of water use would include bathing restrictions on a transient basis, construction of dikes etc. Controls of the product would include chlorination or other methods of disinfection of water used for drinking water supply, treatment plants for contaminated shellfish, and distribution of high-quality bottled water during emergency. Water hyacinth and other oceanic weeds are utilized to redesign wastewater treatment tidal ponds and treat substance wastewaters. Water hyacinth can expel even radioactive metals from effluents, which is extremely troublesome something else. As the metals are separated from the effluents, it causes significant cleaning of the emanating. The pH of effluents after treatment has been observed to be in the vicinity of 6.8 and 7.8, which is a perfect range for fresh water. Further, it doesn't enable the algal sprout to create and the BOD and COD are brought down to the level at which the emanating can be permitted to mix with freshwater without causing any terrible impact. The controlled utilization of water hyacinth in conjunction with waste adjustment lakes not just builds the BOD evacuation limit of these frameworks, additionally diminishes the high aggregate suspended solids ordinarily connected with sewage lagoons. Total estimation of all chemicals (organics& in-organics) in the water/waste water is known as Chemical Oxygen Demand. The measure of oxygen that requires for the microbes to debase the natural segments introduce in water/squander water is known as BOD. COD is constantly higher than BOD and the proportion may even surpass 2.5 relying on the sort of water or wastewater (Henze et al. 2001). Nanoparticles were also used to control water pollution.



Fishes died due to water putrifaction



Water polluted due to industrial waste

Fig 2: Showing effects of water pollution

Nanotechnology is the creation, control and utilization of materials at the nanometer measure scale (1 to 100 nm) (Fatema 2017). Metallic nanoparticles have distinctive physical and chemical properties that exhibit capturing in various modern applications (e.g. higher particular surface zones, particular optical properties, mechanical strengths). Material having measurements in the nanometer range (<100 nm), nanotechnology likewise manages them. Nanoparticles are viewed as answer for some innovation and ecological difficulties. Green synthesis of nanoparticles decreased the risks for worldwide (Dahl et al. 2007). Implantation of these practical procedures ought to receive the essential standards of green science. Diverse compound nature of nanoparticles, including metals, metal oxides, non-oxides, carbon, and biomolecules. Nanoparticles have two or three specific morphology structures, for example, circles, barrels, platelets, tubes, and so forth. NPs physical and characteristic properties vary in a general sense from the properties of atom and mass material. There are chiefly two classes of nanoparticles: natural and inorganic nanoparticles.

Natural nanoparticles are strong particles and are made out of natural mixes which are predominantly lipids or polymeric and may go in distance across from 10 nm to 1m. These may incorporate carbon nanoparticles (fullerenes). Inorganic display novel physical properties. Inorganic nanoparticles may incorporate attractive nanoparticles, metal nanoparticles like gold (used in thermo treatment of organic targets and furthermore as medication carriers) and silver nanoparticles like zinc oxide and titanium dioxide which are semiconductors. Because of the measure of inorganic nanoparticles and their accessibility in compound imaging drugs operators and medications there is a

developing enthusiasm for inorganic nanoparticles (Caseri 2008; Xu et al. 2006). Most present nanomaterials could be sorted out into four sorts:

Carbon Based Materials: These are made generally out of carbon, most usually appearing as an empty circles, ellipsoids, or tubes. Round and ellipsoidal carbon nanomaterials are alluded to as fullerenes, while barrel shaped ones are called nanotubes. **Metal Based Materials:** incorporate quantum specks, nanogold, nanosilver and metal oxides, for example, titanium dioxide (Amin et al. 2014). Dendrimers are nano sized polymers worked from stretched units. Composite join nanoparticles with different nanoparticles or with bigger, mass sort materials. Nanoparticles, for example, nano sized dirt, are as of now being added to items running from car parts to bundling materials, to improve mechanical, warm, hindrance, and fire resistant properties.

The huge particular surface territory of nanoparticles including: the starting point of some of their one of a kind applications (Amaravathi 2015). Nanoparticles and nanostructure are ended up being making in helpful application, including imaging or the transport of medicine to cells, tissues and organs. Numerous pharmaceutical stacked nanoparticles are works together with organ and tissues and are taken up by cells. The nanoparticles are in a network that gives high surface range to the response is initiated by daylight and can work in water (Zhang et al. 2008). Nanoparticles use in different applications, for instance, nano and UV simulated, nano air channel, nano hostile to maturing cream, nano b-12 vitamin shower, nanosack, nano frosty impetus air purifier, teeth cleaner, nano pacifier, nanoclusters.

1.3 Green synthesis of nanoparticles:

Approach of biosynthesis of nanoparticles is a type where the primary response happening is reduction/oxidation. The requirement for biosynthesis of nanoparticles as the physical and chemical procedures was expensive. Usually, synthesis of chemical compounds results in a portion of the harmful compound consumed at first glance that may have an antagonistic impact in the therapeutic applications. This is not an issue with regards to biosynthesized nanoparticles by means of green synthesis. So, in the look of less expensive pathways for nanoparticles blend, researcher utilized microbial proteins and plant extract (phytochemicals). With their cancer prevention agent or lessening properties, they are generally in charge of the diminishment of metal mixes into their individual nanoparticles. Green combination gives headway over chemical and physical technique as it is financially supportive, condition well disposed off in the environment, effectively scaled up for substantial scale. In this method of green synthesis there is no need to use high pressure, energy, temperature and toxic chemicals.

Benefits of green synthesis: Natural strategies have accordingly cleared the path for the "greener synthesis" of nano-particles and these are better techniques due to slower energy. They offer better control over unique development and their modification. This has motivated various nano technological applications for an upsurge in research that enable better control of shape and size on the synthetic routes. The utilization of natural materials like plant extracts, microscopic organisms like bacteria fungi and yeast and proteins for the production of silver nanoparticles offer various advantages of eco-friendly and similarity for pharmaceutical and other biomedical applications as they don't utilize toxic chemicals for the protocol of synthesis (Jung et al. 2006). Different type of methodologies were manageable for the production of silver nanoparticles in particular degradation in various solutions, synthetic and photochemical responses in micelles, thermal disintegration of silver compounds, radiation aided, electrochemical, microwave helped synthesis and by means of green science course (Ramya and Subapriya 2012) .

1.4 Source:

Ziziphus mauritiana is a little estimated subtropical tree, prominent for its little berry like organic product (Seth 2003). It develops wild in woods and furthermore on badlands all through the mid-slopes up to rises of 1,400 meters. A little spreading tree, with hanging branches; tallness, 5 to 8 meters; trunk circumference, 85 cm; bark, harsh, dim or dull dark, sporadically broke, secured with a thick layer of green greenery on account of more seasoned trees and, in this manner, looking green. Leaves, praise, petiolate (petiole, 5 mm long), having fine serration, 3.8 cm long, 2.9 cm expansive, dim green and sparkling from above, white tomentose from underneath (Muchuweti et al. 2005). Trees are effectively proliferated by seeds, notwithstanding, seedlings take a few years to begin natural product creation (Tembo et al. 2008).

1.5 Nutrient content:

This really nutritious natural product is loaded with vitamins and minerals having great amount of vitamin C particularly (Mathur and Vyas 2000). Here is the normal nourishing arrangement of 100gm of Ber or *Ziziphus mauritiana*. Flavonoids are a gathering of plant metabolites that make cell flagging pathways and cancer prevention agent impacts. These particles are found in great amount in Ber natural product. Natural product mash of Ber or *Ziziphus Mauritiana* contains the accompanying flavonoids:

- Epicatechin
- Quercetin-3-O-rutinoside
- Quercetin-3-O-galactoside
- Kaempferol-glucosyl-rhamnoside
- Procyanidin B2

Nutrient content	Quantity(gm)
Carbohydrates	17
Protein	0.8
Fat	0.07
Sugar	10.5
Minerals	
Calcium	6
Phosphorus	6
Iron	0.15
Potassium	70
Sodium	1
Zinc	0.01
Magnesium	3
Vitamins	
Carotene	0.021
Thiamine	0.024
Riboflavin	0.038
Niacin	0.087
Citric acid	1.1
Ascorbic acid	75

CHAPTER 2

REVIEW OF LITERATURE

There is an expanding interest of silver nanoparticles because of their novel properties and applications in different fields, for example, medication, catalysis, nanoelectronics, material field, contamination and water treatment. Nanosilver solid antimicrobial movement is the significant explanation behind the improvement of nanosilver containing items. The silver nanoparticles are synthesized by different chemical and physical techniques (Madhumitha et al. 2016). The real downsides of synthetic and physical strategies are the costly procedures and numerous dangerous chemicals are utilized. To overcome these issues Green Nanotechnology comes to assume exceptionally significant part for mixture of silver nanoparticles. Utilization of different plants for combination of biogenic silver nanoparticles suggests as Green Nanotechnology. In the present investigation, we detailed the green synthesis of silver nanoparticles utilizing by *Ziziphus mauritiana* fruit extract in the presence of daylight climate without utilizing any compound added substance (Khan et al. 2015). The *Ziziphus mauritiana* natural product separate go about as reducing operator and in addition topping specialist. Synthesized AgNPs were properly described and tried for their antibacterial action. The settled AgNPs were then characterized by the UV-Vis spectroscopy; Dynamic light scattering (DLS); Zeta potential and Fourier change infrared (FTIR) spectroscopy. The green synthesized silver nanoparticles show exceedingly antibacterial property against gram positive and gram negative organisms when attempted (Raffi et al. 2008). Progression of green nanotechnology is creating eagerness of masters toward eco friendly biosynthesis of nanoparticles. In this examination, biosynthesis of stable silver nanoparticles was done using *Ziziphus mauritiana* natural fruit extract. Attachment of bioreduced silver nanoparticles was destitute down using UV-vis ingestion spectra, and their antimicrobial development was screened against both gram-negative and gram-positive microorganisms (Tarasenko et al. 2006). It was seen that *Ziziphus mauritiana* natural item focus can reduce silver particles into nanoparticles inside 15 min of reaction time. The resulting nano materials were characterized by UV-visible spectrophotometer, and transmission electron microscope. The UV-Vis spectroscopy revealed the formation of AgNPs at 400–450 nm. Antimicrobial activities of AgNPs were determined by using two bacteria (Gram positive *Bacillus subtilis* MTCC-3160) and Gram negative *Escherichia coli* MTCC- 450) disc diffusion method. The zone of inhibition seems extremely good, showing relatively large zone of inhibition in *Bacillus subtilis* and *Escherichia coli*.

2.1 Silver nanoparticles synthesis using *Ziziphus mauritiana*:

We showed measure controlled mixture of silver nanoparticles (AgNPs) utilizing *Ziziphus mauritiana* natural fruit extract from aqueous arrangement of silver nitrate. The size appropriation of the subsequent AgNPs were observed to be reliant on the convergence of fruit product concentrate and response time, at a similar grouping of silver nitrate(Sutradhar and Saha 2016). The AgNPs were described by UV-Visible spectroscopy (UV-Vis), molecule estimate examination (DLS), Zeta Potential and FTIR. The sizes of the AgNPs were found to diminish with the expansion of centralization of fruit product separate from 300nm to 70nm. The expansion in response time from 5min to 20min altogether diminishes the measure of the AgNPs from 350nm to 100nm.

The natural product is eaten crude, salted or utilized as a part of refreshments. It is very nutritious and rich in vitamin C. It contains 20 to 30% sugar, up to 2.5% protein and 12.8% carbohydrates. The organic products are connected on cuts and ulcers; are utilized in aspiratory infirmities and fevers; and, blended with salt and bean stew peppers, are given in acid reflux and biliousness. The dried ready natural product is a gentle diuretic. The seeds are soothing and are taken, at times with buttermilk, to end queasiness, retching, and stomach torments in pregnancy. They check looseness of the bowels, and are used ointment on wounds. Blended with oil, they are rubbed on rheumatic ranges (Jeong et al. 2005).

2.2 Origin of problem:

The issue of natural contamination has turned into a hot issue in this day and age. Ecological contamination, principally caused by lethal chemicals, incorporates air, water, and soil contamination. This contamination comes about in the pulverization of biodiversity, as well as the debasement of human wellbeing. Contamination levels that are expanding step by step require better improvements or innovative revelations promptly. Nanotechnology offers many points of interest to enhance existing ecological advances and make new innovation that is superior to current innovation. In this sense, nanotechnology has three fundamental capacities that can be connected in the fields of condition, including the cleanup (remediation) and filtration, the discovery of contaminants (detecting and recognition), and the contamination anticipation (Aazam and Zaheer 2016). Nanotechnology offers the capacity to control matter at the nanoscale and make materials that have particular properties with a particular capacity. Nanomaterial is little and the proportion of surface zone to volume proportion is high with the goal that it can be utilized to recognize exceptionally delicate

contaminants. Nanotechnology is additionally used to keep the development of poisons or contaminants by applying the material innovation, mechanical procedures and others.

2.3 Nanotechnology for clean water:

Just 30% of all water on the Earth is not caught in the ice or ice sheets and just 0.08% of it is perfect water, a similarity of 1 teaspoon of water versus a 5 liter holder of water. Lately, water has turned into a critical issue, and it is very hard to take care of the related issues. The advancement of nanotechnology can be utilized to enhance water quality. A few techniques that can utilize nanotechnology utilize receptive media for partition and filtration, bioremediation and sterilization. Remediation is the procedure to evacuate, limit or kill the water contaminants that can harm human wellbeing or biological communities (Theron et al. 2008). Remediation innovations can be isolated into three classifications, to be specific (1) warm, (2) physicochemical and (3) organic techniques. Most customary techniques, for example, extraction, adsorption and oxidation are less successful, costly and tedious, though all earth neighborly organic debasement is modest, yet extremely tedious. A propelled technique that can be utilized is nanomaterials, with upgraded liking, limit and selectivity for overwhelming metals and different contaminants. The upsides of utilizing nanomaterials are their higher reactivity, bigger surface contact and better transfer ability. There are a few cases of nanoparticles and nanomaterials that can be utilized for remediation of water, e.g. zeolites, carbon nanotubes (CNTs), self-collected monolayers on mesoporous underpins, biopolymers, single-chemical nanoparticles, nanoparticles of zero valent iron (ZVI), among others.

A typical framework that has been produced throughout the years to remediate water is known as a 'pump and treat' framework. The framework is intended to direct water from the dirt to the surface and after that to infuse it once more into the ground. Until 1998, the pump and regard framework was as yet utilized as an approach to remediate water. Another approach to remediate water is by utilizing porous responsive boundary (PRB). PRB cleans subsurface groundwater and remediate without the need to convey the water to the surface. This treatment can be utilized to tidy up poisons, for example, chlorinated hydrocarbons, sweet-smelling nitro mixes, polychlorinated biphenyls (PCBs), pesticides and chromate mixes.

Bioactive nanoparticles for water disinfection: Nanotechnology gives an option answer for clean germs in water, an issue that has been deteriorating because of the populace blast, developing requirement for clean water and rise of extra contaminations. One of the choices offered is antimicrobial nanotechnology expressed that few nanomaterials demonstrated solid antimicrobial properties through different instruments, for example, (1) photocatalytic creation of receptive oxygen

species that harm cell segments and infections (e.g. TiO₂, ZnO and fullerol), (2) trading off the bacterial cell envelope (e.g. peptides, chitosan, carboxyfullerene, CNTs, ZnO and silver nanoparticles), (3) interference of vitality transduction (e.g. Ag and fluid fullerene nanoparticles) and (4) restraint of chemical action and DNA union (e.g. chitosan). Among all materials, TiO₂ has been proposed to be the best competitor as it is steady in water, nontoxic when ingested and ease.

Nanofibres and Nanobiocides for water purification: Nanofibres and nanobiocides give a probability to enhance the nature of water filtration layers. For film fouling caused by microorganisms in the water which diminish the nature of water, hindrance of these microscopic organisms can be caused by the surface-altered nanofibres. In view of du Plessis' outcome, both polyvinyl liquor (PVA) and polyacrylonitrile (PAN) nanofibres containing silver nanoparticles have phenomenal antimicrobial movement, with PVA nanofibres diminishing in the vicinity of 91% and 99% of microorganisms in a polluted water test and PAN nanofibres slaughtering 100%. Neither PVA nor PAN nanofibres filtered silver into the water, as it was inferred that PVA is a nontoxic and biodegradable engineered polymer and PVA–silver nanofibres have amazing antimicrobial movement (Li et al. 2008).

The water decontamination prepare utilizing nanotechnology can utilize press nanoparticles, ferritin, polymeric nanoparticles, nanofibres, nanobiocides, nanoenzymes and nanofiltration procedures. Notwithstanding being connected in cleaning and water refinement, nanotechnology can likewise be connected to clean the air from lethal gasses, for example, CO, VOCs and dioxins utilizing CNTs, gold nanoparticles and different adsorbents(Albrecht et al. 2006). Nanoparticles and nanotubes can likewise be connected as a sensor for poisonous substances, especially substances that are hard to recognize with ordinary innovation since they have a little in size and focus.

RESEARCH GAP

Using *Ziziphus mauritiana* extract silver and gold nanoparticles were synthesized by researchers. Gold *Ziziphus mauritiana* extract nanoparticles are used for various applications like dye degradation, pesticide degradation etc. But AgNPs synthesized using *Ziziphus mauritiana* extract had never been used for different applications. In present work, using *Ziziphus mauritiana* extract, AgNPs were synthesized and their applications were studied such as Dye degradation, antibacterial activity, pesticide degradation. From all the above observations, it can be concluded *Ziziphus mauritiana* AgNPs synthesized can be used in the process of water purification and shows better results in comparison to gold nanoparticles.

OBJECTIVES

The principal objective of this research work is:

- Synthesis of AgNPs from silver salts using biological route
- Characterization of synthesized silver nanoparticles
- Determination of the size and shape of AgNPs
- Study of its antimicrobial activity against a few common bacteria
- Adsorption of cationic and neutral dyes
- Reduction of pesticides with temperature and time dependent parameters

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Material Collection:

Ziziphus mauritiana natural product was gathered from the neighborhood market and washed completely with refined water to expel the clean particles. The altogether washed natural product was cut into little pieces. These little pieces at that point dried in nearness of daylight for 6-7 hours.

3.1.1 Preparation of *Ziziphus mauritiana* Extract:

Around 4g of these finely measured natural product pieces were weighed and moved into 250mL container containing 100mL distilled water and boiled for around five hours. The concentrate was then sifted through Whatman No: 1 channel paper to expel particulate issue and to get clear arrangement (ZAE) and put away in fridge which to be utilized for future works.

3.1.2 Preparation of Silver Nitrate Solution:

Stock solution of silver nitrate was set up by including 17mg of silver nitrate in 100ml of refined water. This arrangement is kept secured and wrapped with aluminum wrap in dull so as keep away from its reduction.

3.1.3 Synthesis of Silver nanoparticles at different pH:

Ziziphus mauritiana was taken in 5X fixation and after that its pH was set at various pH i.e. 3, 5, 7, 9, 11, 13. AgNO₃ (240 μM) 1,440 μl was included 3560 μl of ZAE. 5 ml of *Ziziphus mauritiana* remove each from above various pH was taken and afterward included into 1,440 μl of silver nitrate arrangement and kept in coordinate daylight climate for lessening of Ag⁺ particles into AgO. The total diminishment of AgNO₃ was affirmed by the adjustment in shading because of the surface Plasmon resonance.

3.2 Reagents and chemicals:

Ziziphus mauritiana, Silvernitrate, Bacterial cultures i.e. *E.coli* (MTCC77) and *B.subtilis*, Luria broth, Methlene Orange, Alizarine, Agar, Paradol, Nitrophenol, Sodium borohydride, Sodium hydroxide, Hydrochloric acid.

3.3 Instrumentation:

3.3.1 Magnetic Stirrer:

A magnetic stirrer of REMI 2MLH is a laboratory device which uses magnetic field to mix liquid samples, since only a small magnetic bar has to put inside the liquid sample to start the process of mixing. It is used in the experiment for the preparation of clay slurry and also for the mixing of Zinc oxides with the solution. Magnetic stirrer often includes a hot plate or some other means for heating the liquid.

3.3.2 Weighing Balance:

Accurate quantities of used chemicals can be achieved with the help of weighing balance (SARTORINS)

Maximum-250gm; Density-0.01mg

3.3.3 Laboratory centrifuge:

Centrifugation is a process that involves use of centrifugal force for the sedimentation of heterogeneous mixtures. This process is used to separate two immiscible liquids. The laboratory centrifuge (PHYSILAB) works under the principle where the centripetal acceleration will cause denser substances to move outward in the radial direction, the substances which are less dense are displaced and move to the centre.

3.3.4 Photochemical reactor:

The photochemical reactor of PERFIT is used for the degradation of dye. The photochemical reactor used in the experiment needs no water cooling. The normal operating temperature is approximately 35°C with the fan and without the fan it is approximately 60-70°C. No dangerous high voltage is required and intense source of these reactors is of ultraviolet light approximately 1.65×10^3 photons/sec/cm² at 2537 Å. The reactor comes complete in a ready to use package with no assembly required. The UV -lamp has long life of about 3000 hours and is versatile, cheap with the power consumption of 400 watts.

3.3.5 Autoclave:

An autoclave is a pressure chamber used to do modern procedures requiring hoisted temperature and pressure not the same as encompassing pneumatic stress. Autoclaves are utilized as a part of therapeutic applications to perform sanitization and in the substance business to cure coatings and vulcanize elastic and for aqueous union. Many autoclaves are utilized to disinfect hardware and supplies by subjecting them to high-weight immersed steam at 121 °C (249 °F) for around 15–20 minutes relying upon the span of the heap and the substance.

Schematic diagram representing synthesis of silver nanoparticles from *Ziziphus mauritiana* extract:

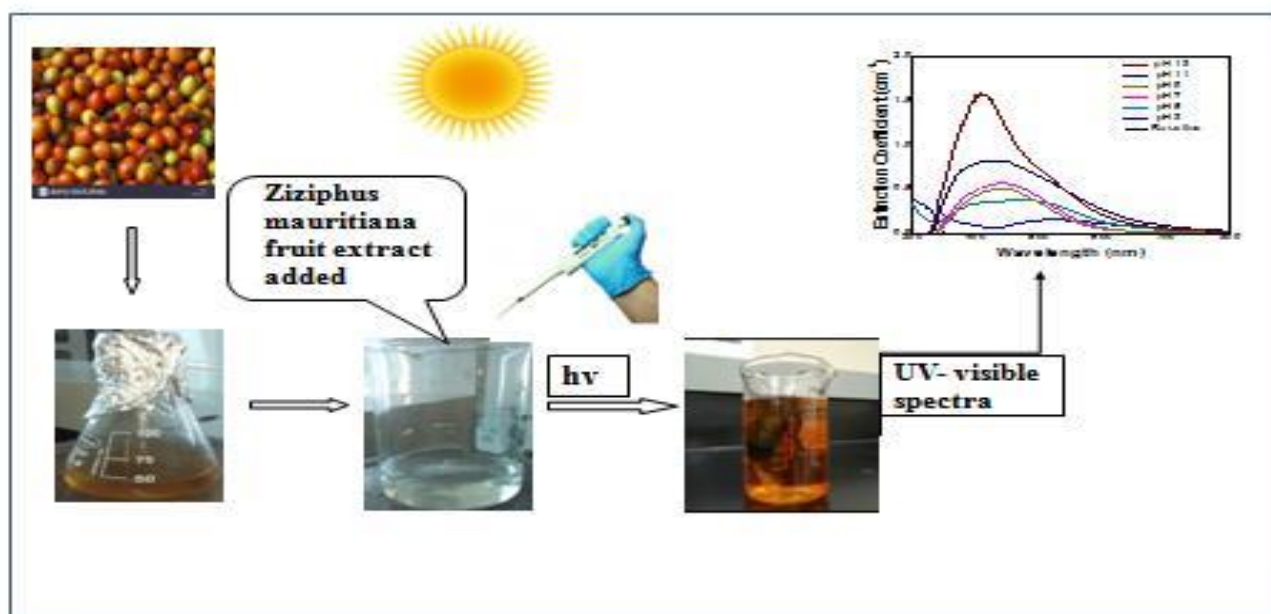


Fig 3: Graphical representation showing synthesis of silver nanoparticles using *Ziziphus mauritiana* extract

3.4 Characterization:

For characterization of the Green silver nanoparticles following methods were done by means of which we can confirm the production of Silver nanoparticles; we can have an idea of its size distribution profile and surface morphology and above all, we can determine the actual particle size as well. The following instruments were used to characterize the green silver nanoparticles that we synthesized biologically.

- UV-vis Spectroscopy
- Fourier Transform Infrared Spectroscopy (FTIR)
- Dynamic Light Scattering (DLS)
- Zeta Potential

3.4.1 UV-vis spectroscopy:

After synthesis of ZAE protected AgNPs UV-visible spectral absorbance was measured using Analytic Jena UV-Visible spectrophotometer, operated between 1100 to 200 nm wavelength ranges. Ultraviolet and visible adsorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample. Absorption measurements can be at a single wavelength or over an extended spectral range. The reduction of Ag⁺ ions was confirmed by UV-vis spectrophotometer. The addition of *Ziziphus mauritiana* fruit extract to silver nitrate solution resulted in color change of the solution due to the production of silver nanoparticles. These color changes arise because of the excitation of surface plasmon vibrations with the silver nanoparticles. To observe the optical property of biosynthesized silver nanoparticles, samples were periodically analyzed for UV-vis spectroscopic studies at room temperature operated at a resolution of 1 nm between 250 nm and 700 nm ranges. The spectral peak of the silver nanoparticles prepared at all pH by green synthesis lies between the range 420-480nm.

3.4.3 Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR uses interferometer to record information about a material placed in the IR beam. Then Fourier Transform results in spectra that analysts can use to identify or quantify the material.

- An FTIR spectrum arises from interferograms being ‘decoded’ into recognizable spectra
- Patterns in spectra help identify the sample, since molecules exhibit specific IR fingerprints.

For Fourier transform infrared spectroscopy (FTIR) measurements, the reaction mixture was centrifuged at 15,000 rpm for 15 min to separate Ag nanoparticles from biomass or other bioorganic compounds after complete reduction of AgNO₃ by the *Ziziphus mauritiana* fruit extract. The Ag nanoparticles pellet acquired after centrifugation were re-scattered in water and washed (centrifugation and re-scattering) with distilled water for three times. At long last, the specimens were dried and pounded with KBr. FTIR study imparts the biomolecules responsible for Ag⁺ ions and stabilization of silver nanoparticles. The FTIR analysis in the range of 4000-400 cm⁻¹ was carry out to examine the presence of capping agent and role of molecules involved in the synthesis of silver nanoparticles.

3.4.4 Dynamic Light Scattering (DLS):

Dynamic Light Scattering is utilized for measuring the span of particles normally in the sub micron locale, likewise liked to as Photon Correlation Spectroscopy. It takes a shot at the rule that particles suspended inside a fluid experience Brownian motion. It is based on the laser diffraction

method with multiple scattering technique was employed to study the average particle size of silver nanoparticles. The bigger the molecule, the slower the Brownian movement will be DLS screens the Brownian motion with light disseminating. It gives us information about size profile and stability of nanoparticles in solution. In DLS, the speed at which the particles are diffusing due to Brownian motion is measured by recording the rate at which the intensity of the scattered light fluctuates. Smaller particles cause the intensity to fluctuate more rapidly than large particles.

3.4.5 Zeta Potential:

A potential exists between the molecule surfaces and scattering fluid which changes as indicated by the separation from the molecule surface is known as zeta potential. It is utilized to decide if the molecule inside a fluid will have a tendency to flocculate or not. Stability of ZM AgNPs was monitored spectroscopic ally by using Malvern v2.3 Zeta potential analyzer.

3.5 Applications of *Ziziphus mauritiana* AgNPs:

3.5.1 Antibacterial activity:

E.coli and *B.subtilis* were taken. Microorganisms were kept up on agar plates. Colonies were set up by inoculating 400 ml Luria broth (LB) with single settlement of every bacterium taken from agar plate. Broths were inoculated overnight at 35°C with shaking.

The antibacterial activity of green synthesized nanoparticles was studied by batch culture method. Aqueous dispersions of silver nanoparticles of various concentrations (0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.50, 0.75, and 1.0) were made. Silver nanoparticles of seven different pH i.e. pH 3,5,7,9,11 and 13 were taken .For each pH, set of nine test tubes were taken .With different concentrations of nanoparticles were taken ,then stock culture containing *E. coli* and *B.subtilis* were added and cultured separately in liquid medium (Luria broth). Required measure of agar medium was set up alongside silver nanoparticles of fixation 100µg/ml. Another agar media without silver nanoparticles was likewise arranged which was utilized for refined strains without nanoparticles. Both were autoclaved and filled diverse test tubes. The above strains were refined. The cultures without nanoparticles were kept as control for a correlation consider. General 5 ml volume was kept up by utilizing refined water. Test tubes were kept for overnight blending at 37 °C .at that point absorbance was taken at 600 nm. Same technique was completed for *B. subtilis*.

3.5.2 Dye degradation:

Precise quantities of solid dyes (Methylene Orange and Alizarin each 50 mg in 100 ml) were dissolved in double distilled water to prepare stock solution of reactive dyes. Solutions of various concentrations of dye were obtained by diluting this stock solution (1 ml of each solution in 50 ml of distilled water) and making it as working solution. The process was carried out using all the seven pH (pH 3, 5, 7, 9, 11, 13). In 5 ml of *Ziziphus mauritiana* AgNP solution of each pH, 100 µl of working solution of each dye at all pH was added and kept for incubation in dark with stirring at 30°C. The readings were taken with UV-Vis spectrophotometer at every 10 minutes. The Methylene Orange showed its peak at 460 nm and Alizarin was observed at 600 nm.

3.5.3 Adsorption of Pesticides:

The pesticide used for adsorption here is parathion (Methyl parathion). This experiment was carried out in 2 different ways:

1. Time ward adsorption
2. Temperature ward

Stock arrangement was set up by including 40 mg of pesticide in 100ml of twofold refined water. For time-subordinate clump 2.5 ml *Ziziphus mauritiana* AgNP was taken and 100 µl of pesticide arrangement was added to it and kept for hatching in dim for mixing at 30°C. Then readings were taken at UV-vis spectrophotometer after 0 hr, 1 hr, 3 hr, 6 hr and 24 hr. The peak was seen at 400 nm. Over 90% of the response is finished inside 15 minutes of response time.

3.5.4 Nitrophenol reduction:

Kinetic studies for reduction of 4-NP is done using homogenous catalyst at different pH and time intervals. Freshly prepared 0.01 M NaBH₄ is added to 10⁻²M 4-NP. UV spectra were taken, after that catalyst (*Ziziphus mauritiana* AgNP) at different focus and pH was included and absorbance was taken after at regular intervals.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of NPs and color change with sunlight

During the synthesis of silver nano particles change in color has been observed in the Figure. Silver nitrate which was used as starting material was initially yellow in color. When fruit extract was added to silver nitrate solution then the color immediately changes from yellow to brownish color. When further exposed to sunlight color changes to dark brownish. Synthesis of silver nanoparticles at different time intervals is shown in Fig4.

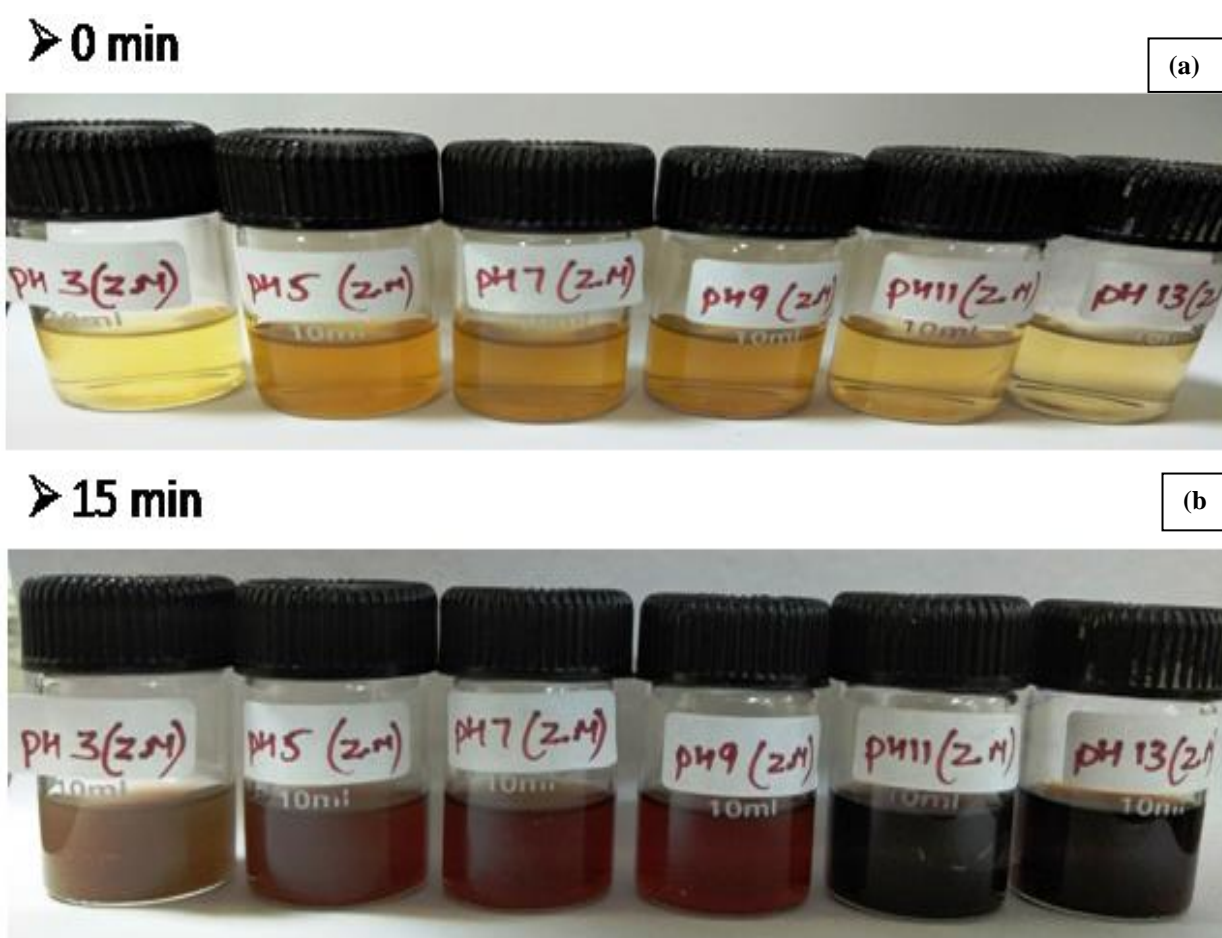


Fig 4: Showing synthesis of AgNP at different time intervals

4.2 UV-vis spectrophotometer:

The synthesis of silver nanoparticles was initially confirmed visually and by using UV-visible spectroscopy technique which has been frequently used to characterize the synthesized metal and metal oxide nanoparticles. Due to surface Plasmon resonance phenomenon there is change in color of

reaction mixture which indicates the formation of silver nano particles in the reaction mixture. The optical properties of silver nanoparticles were investigated by UV-visible analysis. The UV-absorption spectroscopy of the sample shows an absorption peak is at about 440 nm, which confirm the synthesis of silver NPs. It shows the change in color of the reaction medium as an effect of presence of any type of reducing substance. In case of bacteria and fungi mediated synthesis of AgNPs, reduction of silver nitrate to elemental silver has been attributed to the presence of reductive .Graph shows UV-Vis absorption spectrum of silver nanoparticles. Through the plasmon band is wide because of the nearness of segments in *Ziziphus marutiana* separate which are additionally being perused in the spectrophotometric run, it is watched that the silver surface plasmon reverberation (SPR) happens at 430 nm and relentlessly increments in power as a capacity response time. As the pH increases, the size of the nanoparticles decreases. Inset of Figure 5 speaks to the plot of absorbance at λ_{max} versus response time. The reduction

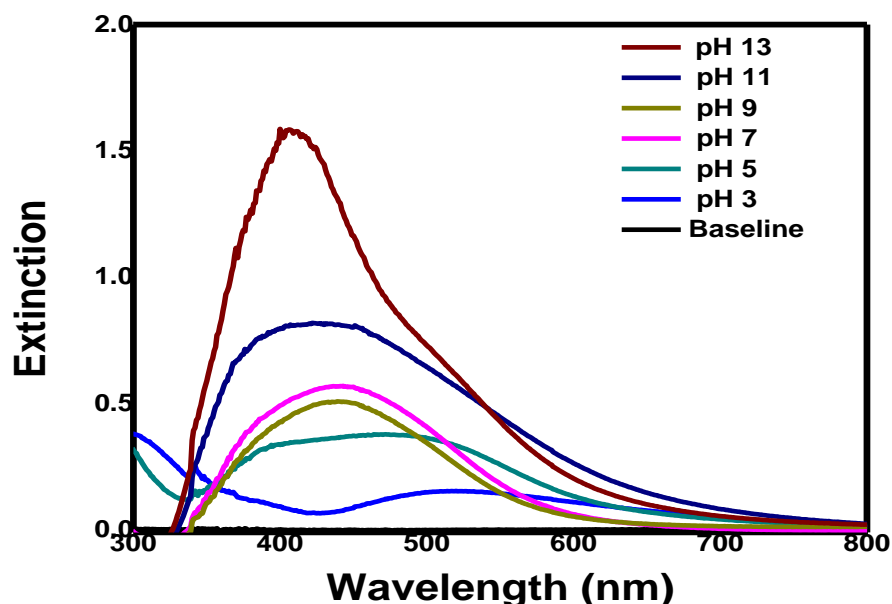


Fig 5: Shows the UV-Vis spectra of silver nanoparticles synthesized

4.3 FTIR:

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive procedure valuable for recognizing natural chemicals in an entire scope of uses despite the fact that it can likewise describe some inorganic incorporate paints, glues, saps, polymers, coatings and medications. The recurrence extend is measured as wave numbers regularly finished the range 4000 – 400 cm^{-1} . For Fourier change infrared spectroscopy (FTIR) estimations, the response blend was centrifuged at 15,000 rpm for 15 min after total lessening of AgNO_3 by the *Ziziphus marutiana* natural product concentrate to

isolate Ag nanoparticles from biomass or other bioorganic mixes which may meddle in dissecting protein AgNPs cooperation. The Ag nanoparticles pellet acquired after centrifugation were re-scattered in water and washed (centrifugation and re-scattering) with distilled water for three times. At long last, the specimens were dried and crushed with KBr pellets and examined. The peak in 1035-1085cm⁻¹ species the presence of C-O bond, 1300-1400cm⁻¹ shows presence of C-H bond(stretch), 1595-1655cm⁻¹ shows N-H bend, 2850-3000cm⁻¹ shows C-H (bend) in alkanes and alkyls, range 3200-3300cm⁻¹ shows O-H (stretch) whereas 3400-3500cm⁻¹ shows N-H symmetric and asymmetric stretch. The data at different ph is observed with comparison to control.

Table 2: Showing bond stretching –bending in FTIR spectrum

Functional groups (cm⁻¹)	C-H bending	O-H bond	C-O-C	C=O&C-N (stretch)	Triple bond C&O and C&N(stretch)	N-H (stretch)	O-H (stretch)
Control	-	-		1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹
pH-3	-	-		1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹
pH-5	-	-		1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹
pH-7	750 cm ⁻¹	872 cm ⁻¹	1111 cm ⁻¹	1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹
pH-9	750 cm ⁻¹	880 cm ⁻¹	1111 cm ⁻¹	1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹
pH-11	750 cm ⁻¹	880 cm ⁻¹	1111 cm ⁻¹	1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹
pH-13	750 cm ⁻¹	877 cm ⁻¹	1111 cm ⁻¹	1523-1712 cm ⁻¹	2320-2400 cm ⁻¹	3611cm ⁻¹	3748-3841 cm ⁻¹

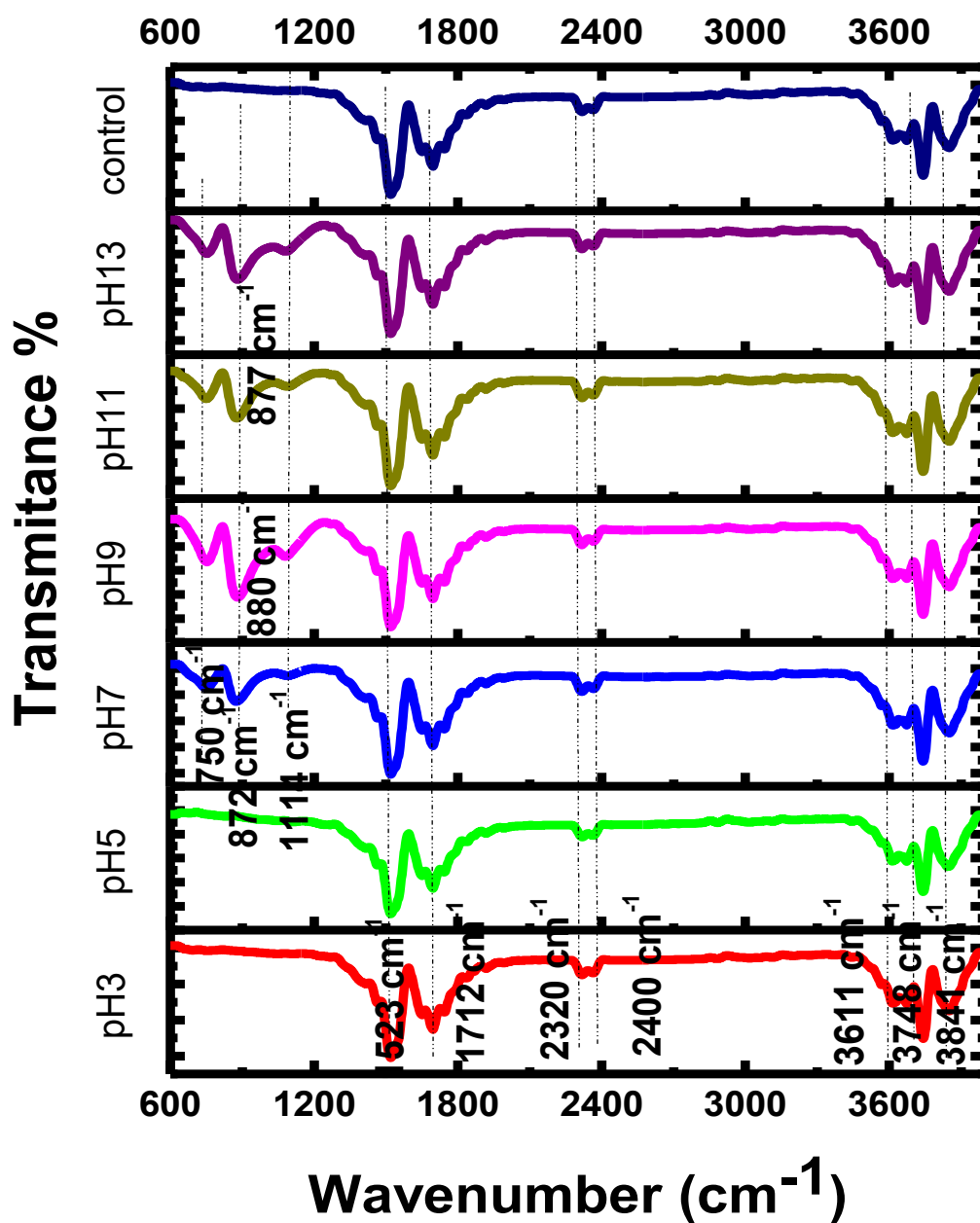


Fig 6: Showing analysis of *Ziziphus mauritiana* AgNPs at different pH in FTIR

4.4 Dynamic Light Scattering (DLS) and Zeta Potential:

Dynamic Light Scattering is utilized for measuring the extent of particles ordinarily in the sub micron area, additionally wanted to as Photon Correlation Spectroscopy. It depends on the laser diffraction strategy with various diffusing method was utilized to ponder the normal molecule size of silver nanoparticles. The bigger the molecule, the slower the Brownian movement will be. DLS screens the Brownian motion with light dispersing. It gives us the data of size profile and steadiness of nanoparticles in arrangement. In DLS, the speed at which the particles are diffusing because of

Brownian movement is measured by recording the rate at which the force of the scattered light changes. Smaller particles make the force change more quickly than substantial particles. Dynamic light scattering or Photon Correlation Spectroscopy is a strategy utilized as a part of material physical science for deciding the size distribution profile of nanoparticles in suspension or polymers in arrangement. Light scattering strategy is utilized here to decide the size distribution profile of nanoparticles display in the last arrangement after ultracentrifugation. From this estimation, the mean size of particles inside the specimen is gotten alongside the relationship between the quantity of particles of a specific size versus the extent of the nanoparticles. The particle size distribution (PSD) of synthesized silver nanoparticles of different pH (3, 5, 7, 9, 11,13) According to figure 7 the colloidal solution of silver nanoparticles of pH 3 ranges at 193nm but in case of pH 13, the solution contains particles of uniform sizes ranging at 8nm. From this measurement, the mean size of particles inside the sample is obtained along with the correlation between the number of particles of a particular size versus the size of the nanoparticles. The particle size in case of pH 5 ranges at 48nm. Similarly the sizes of nanoparticles in case of pH 7 range at 23nm, pH 9 range at 17 nm and pH 11 ranges at 11nm. If we compare the below five results we can conclude that the pH 5, 7, 11 give uniform distribution of particles but pH 3 ranges at 193nm. Among them pH 9 is very appropriate since it gives lowest average size of nanoparticles.

The zeta potential value of *Ziziphus mauritiana* AgNPs has a sharp peak negative value, suggests that *Ziziphus mauritiana* AgNPs has negative charge at the surface; negative charge confirms the repulsion among the nanoparticles and proves that they are very stable and dispersed in medium.

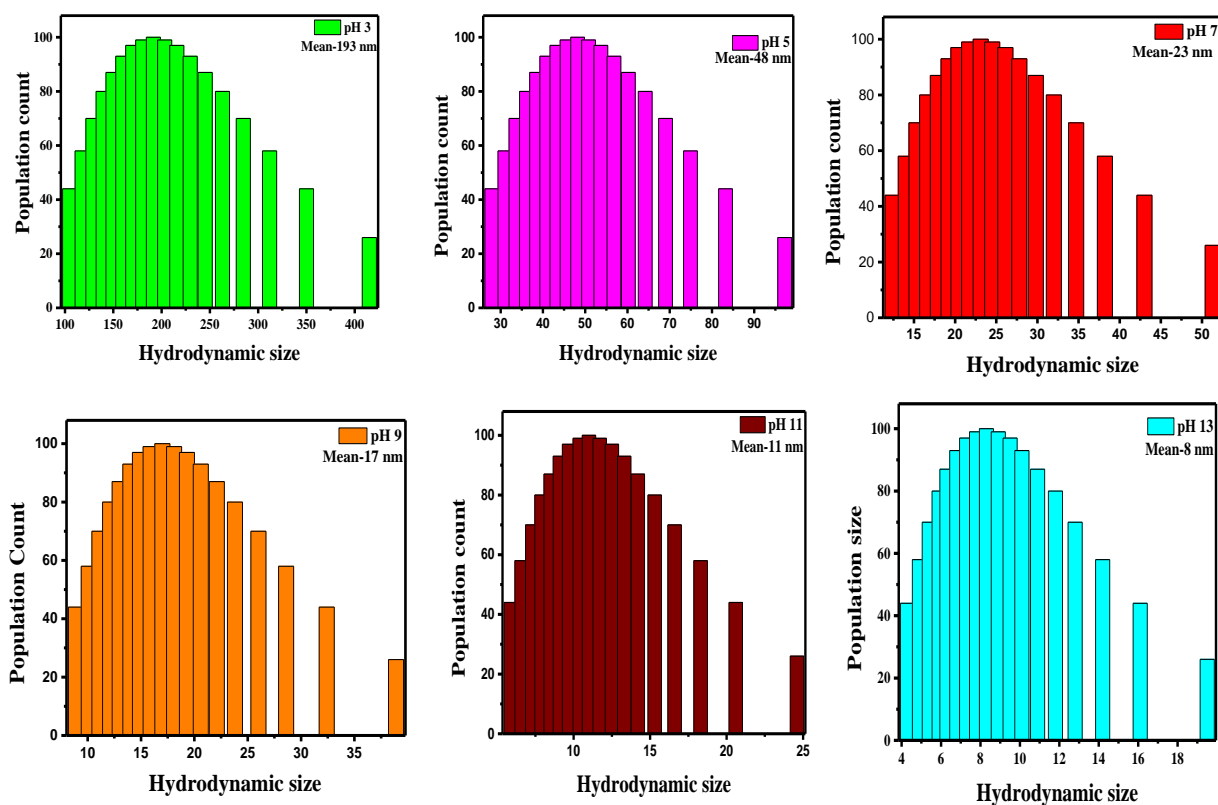


Fig 7: DLS showing comparison of hydrodynamic size of *Ziziphus mauritiana* AgNPs at pH ranges 3, 5,7,9,11,13.

Table 3: Showing with increase in pH particle size decrease.

pH of AgNPs	Hydrodynamic Size (nm)	Zeta potential (mV)
3	193	-10.1
5	48	-15.3
7	23	-17.7
9	17	-15.8
11	11	-23.0
13	8	-17.1

4.6 Antibacterial activity:

The antimicrobial activity of synthesized nanoparticles was studied by batch culture method. Aqueous dispersions of silver nanoparticles of various concentrations (0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.50, 0.75, and 1.0) were made. Stock culture of *E. coli* and *Bacillus subtilis* were grown separately in liquid. The cultures without nanoparticles were kept as control for a comparison study. It is observed from figure 8 (a) and (b), AgNPs shows significant antibacterial activity as compare to control. Also as the pH of the AgNPs increases the antibacterial as activity also increases. The reason behind this may the decrease in hydrodynamic size with increase in pH from 3 to 13 shown by DLS.

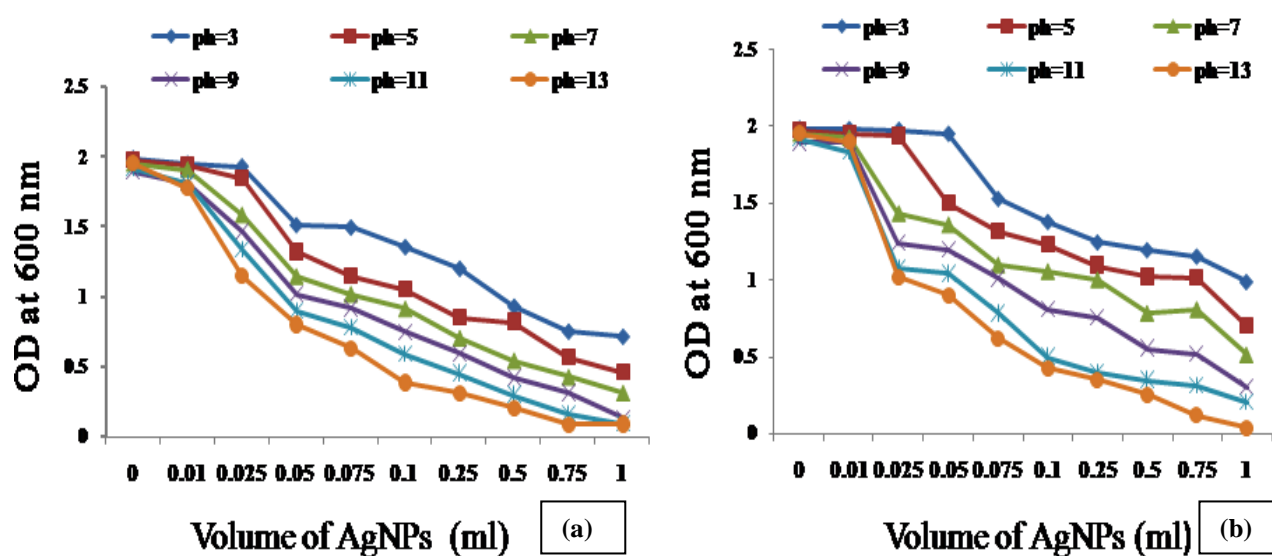


Fig 8: (a) Graph showing growth inhibition of *E. coli* in different pH medium.

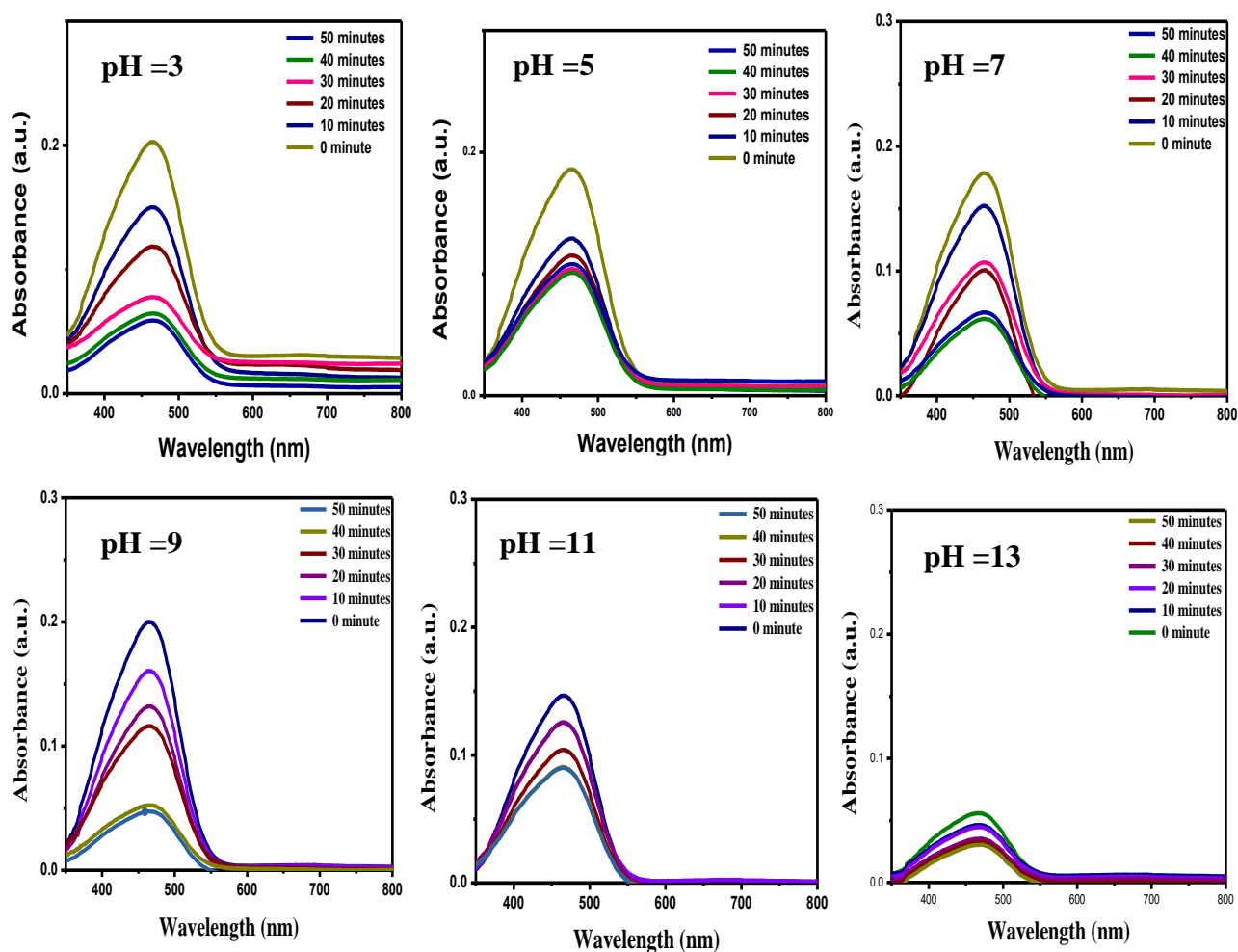
(b) Graph showing growth inhibition of *B. subtilis* in different pH medium.

Table 4: Values showing the effectiveness of a substance

pH	IC50 Value(<i>E. coli</i>) (mg/mol)	IC50 Value(<i>B. subtilis</i>) (mg/mol)
3	7.99	9.77
5	6.60	8.11
7	5.77	6.78
9	5.22	5.64
11	4.72	4.68
13	4.27	4.38

4.7 Adsorption of Dyes:

The figure 9 proves that, after 50 minute of reaction between Methlene Orange and Alizarin with strong reducing agent silver on the addition of silver nanoparticles, degradation of the dye MO is greatly enhanced. The disappearance of blue color of MO and red color disappearance of Alizarin in solution was due to the reduction. The neutral dye n cationic dye solution turns into completely colorless solution within less than 50 minute in dark with vigorously stirred. Similar results show by using the biosynthesized AgNPs using *Ziziphus mauritiana* extract. The plot of relative absorption intensity with wavelength in a regular interval of time reveals that the complete reduction was accomplished in less than 60 min in the presence of *Ziziphus mauritiana* silver nanoparticles.



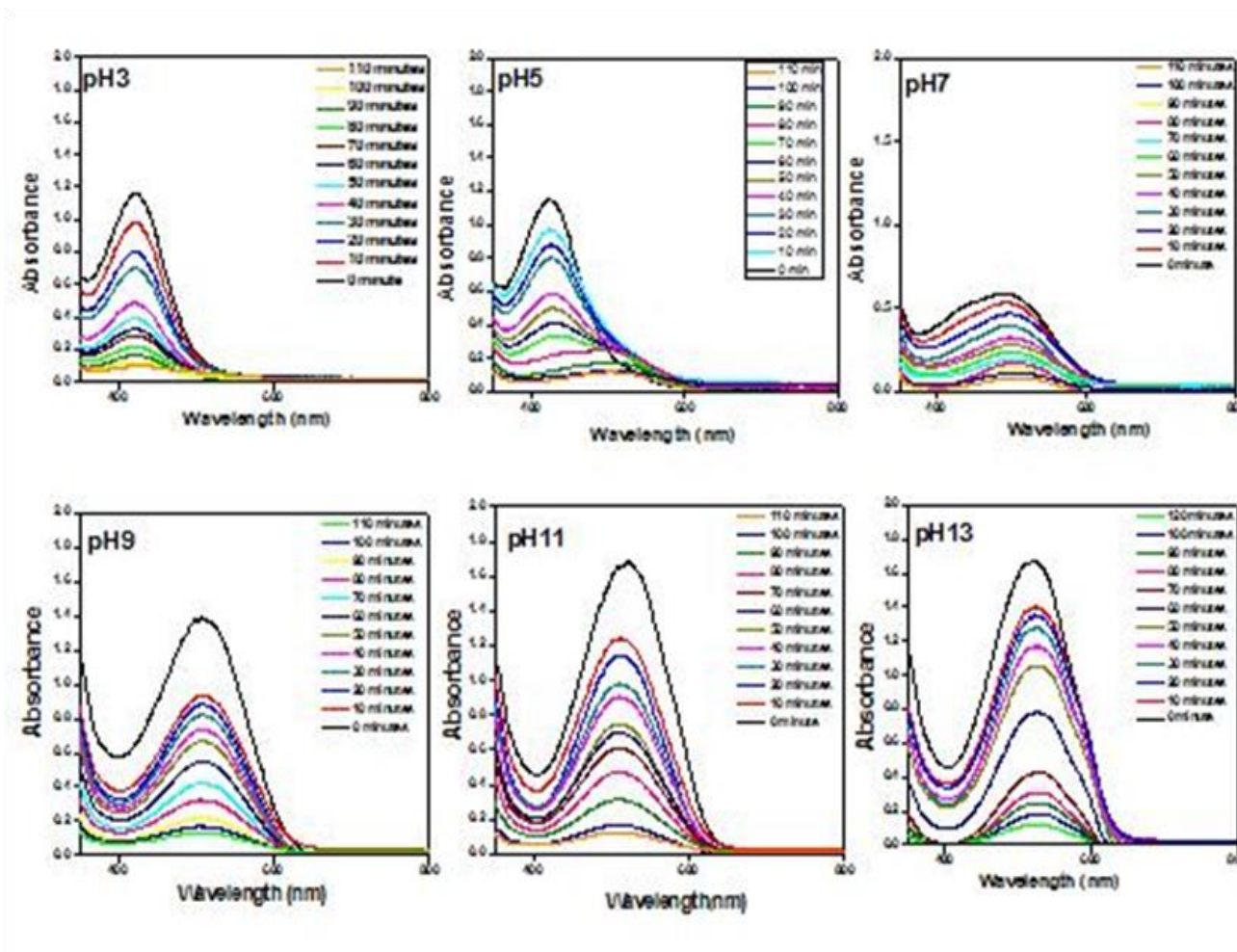


Fig 9: (a) Graph showing reduction of Methylene Orange

(b) Graph showing reduction of Alizarin Red at 110min

4.8 Adsorption of pesticide:

Nanoparticles exhibit a higher surface area-to-volume ratio with a decrease in the size of particles. It has been well established that the metallic nanoparticles such as zero-valent iron, copper, silver and gold have unique catalytic activity.

For time dependent batch solution of *Ziziphus mauritiana* AgNP was taken and to it 100 μ l of pesticide solution was added and kept for incubation in dark for stirring at 300°C. The reading was taken at UV-vis spectrophotometer after every 0hr, 1hr, 3hr, 6hr and 24hr. The peak was observed at 400 nm. Figure 10 showing as the time increases, absorbance decreases.

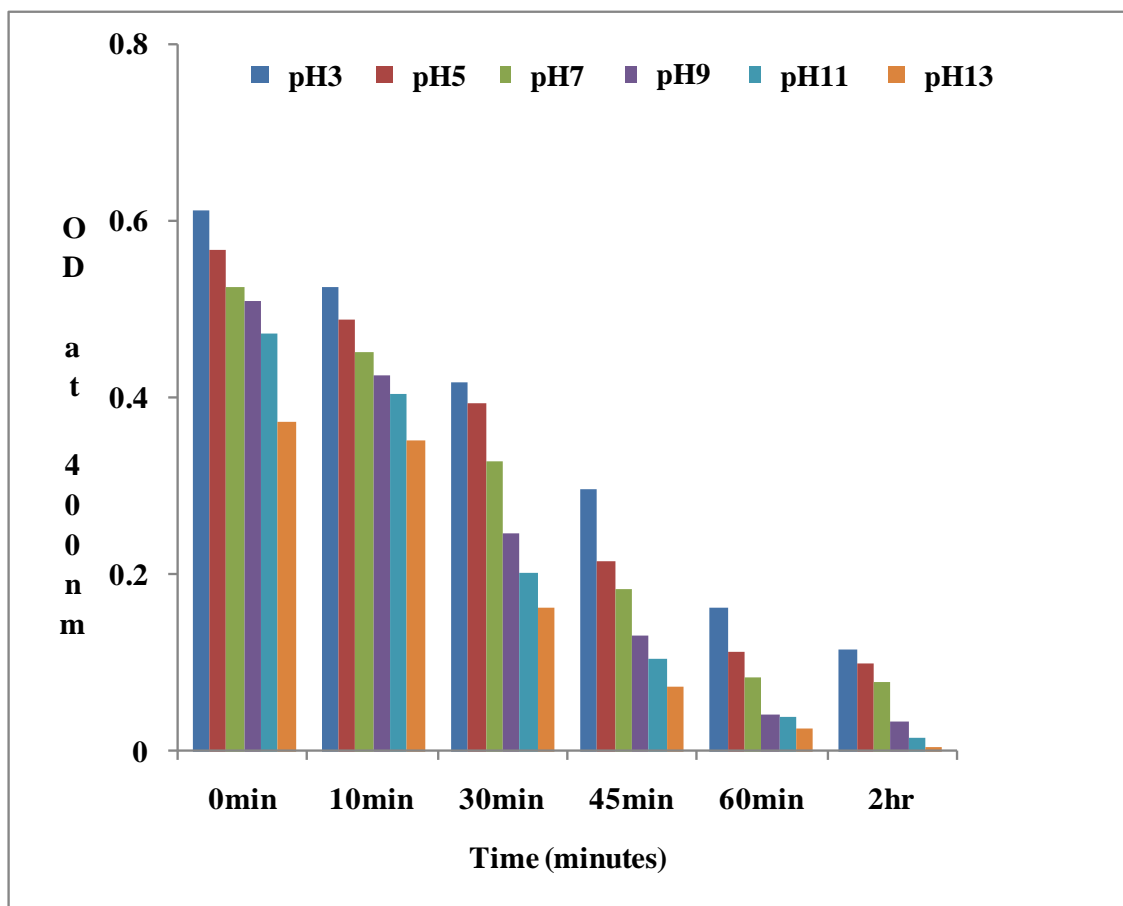


Fig 10: Graph showing time dependent batch at 300°C

4.9 Nitrophenol Reduction:

The resultant UV-vis spectra of the catalytic procedure of 4-nitrophenolate to 4-aminophenolate after adding *Ziziphus mauritiana* silver nanoparticles are shown in Figure11. The main peak of 4-nitrophenolate ions at 400 nm is decreasing with reaction time, while the second peak at 300 nm of 4-aminophenolate is slowly increasing. The time dependence of absorbance of 4-nitrophenolate ions at 400 nm is characterized by an induction period t_0 , wherein the absorbance is constant, followed by a linear decrease.

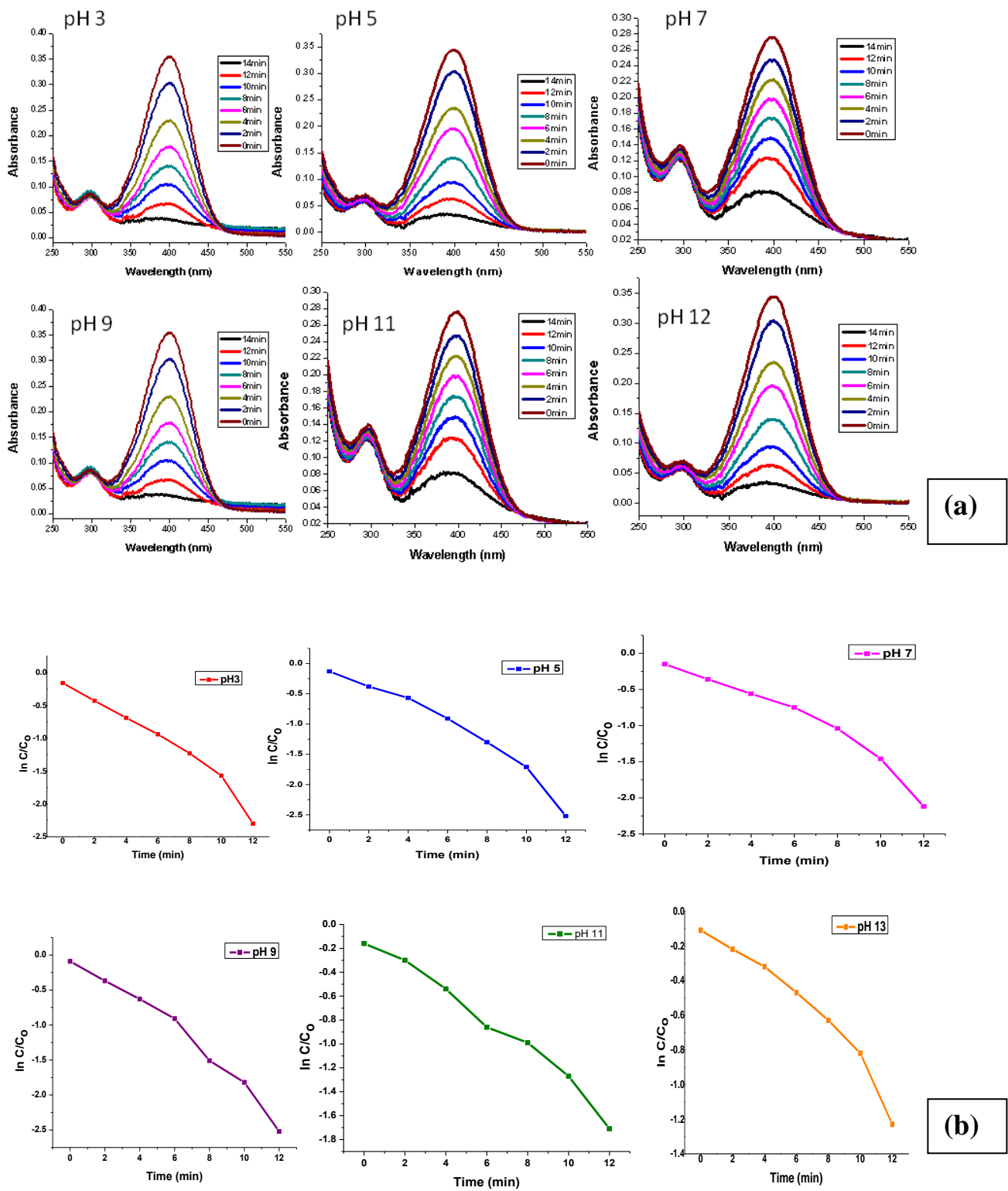


Fig 11: (a) and (b) Graph showing reduction of 4-nitrophenolate and production of aminophenolate.

5. CONCLUSION:

The rapid biological synthesis of silver nanoparticles using *Ziziphus mauritiana* fruit extract provide environmental friendly, simple and efficient route for synthesis of benign nanoparticles was done at different pH medium (3, 5, 7, 9, 11,13). The synthesized nanoparticles were of spherical shaped and the estimated sizes were 10-100 nm at different pH range. The nanoparticles were surrounded by a thin layer of *Ziziphus mauritiana* that contains betulinic aldehyde, betulinic acid, ceanothic acid, frangufoline, spinosin, beta-sitosterol, daucosterol, daucosterol-6'-octadecanoate, sucrose, docosanoic acid, stearic acid, palmitoleic acid flavonoids, carbohydrates, proteins and fats which were found from the characterization using UV-vis spectrophotometer, DLS, Zeta Potential and FTIR techniques. Every one of these systems it was demonstrated that the grouping of natural product concentrate to metal particle proportion assumes an essential part in the shape assurance of the nanoparticles. The higher concentrated nanoparticles had sheet formed appearance where as the lower proportions indicated spherical appearances. The sizes of the nanoparticles in various pH range were additionally unique which rely upon the decrease of the size of metal nanoparticles. From the information of DLS it was discovered that pH 13 had sharp nanoparticles of around 8 nm and whereas few nanoparticles has around 10-800 nm relying upon various pH range. From the mechanical perspective these silver nanoparticles have potential applications in the antibacterial activity, adsorption of various organic dyes and degradation of pesticides and this straightforward strategy has a few favorable circumstances, for example, cost-viability, similarity for therapeutic and pharmaceutical applications and in addition substantial scale business generation.

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