

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *MUNTINGIA CALABURA L*, (*FRUITS*)*CITRUS RETICULATA LEAVES* AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Nanoparticles synthesis has its great impact in recent times due to their advantageous properties and varied applications. Research on green production methods for metal oxide nanoparticles is growing with the objective to overcome the potential hazards of these chemicals for a safer environment. The present work reports a simple, cost effective and eco- friendly method. We have explored an inventive contribution for synthesis of silver nanoparticles using *MUNTINGIA CALABURA L(FRUITS)*, *CITRUS RETICULATA LEAVES*, extract. The procedure for the development of stable silver nanoparticle is rapid and simple. Synthesized nanoparticles were characterized by various methods, such as UV-Vis spectroscopy, FT-IR, SEM, XRD and Antimicrobial activity.

Keywords: *MUNTINGIA CALABURA L*, *CITRUS RETICULATA LEAVES*, Silver nitrate, UV-VIS, FT-IR, XRD, SEM, E.Coli, Klebsiella, S.aureus.

I.Introduction :

Nanobiotechnology is an upcoming branch nanotechnology which has been playing an important role in the field of medical science. Bioelectronics and biochemical applications and it often studies existing elements of living organisms and nature to fabricate new nano-devices. Elucidation of the mechanism of plant-mediated synthesis of nanoparticles is a very promising being simple, eco-friendly and economically viable compared to the microbial systems like bacteria and fungi because of their pathogenicity, and also the chemical and physical methods used for synthesis of metal nanoparticles.

There are many ways to synthesize nanoparticles such as sol gel method, chemical reaction, solid state reaction and co- precipitation. Compared to those methods, green synthesis method is one of the best method for the synthesis of nanoparticles in recent years . This method has several advantages namely low cost, simple, use of

less toxic materials, most important is eco- friendly. In this method, the plant extract has been used as reducing agent for the synthesis of silver nanoparticles .

1.1 Why Silver?

The synthesis of metal nanoparticles is an important topic of research in modern material science. Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. However, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes . In medicines, silver and silver nanoparticles have a application including skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices and implants prepared with silver-impregnated polymers. In textile industry, silver-embedded fabrics are now used in sporting equipment.

1.2 Application of Silver Nanoparticles

Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive and antibacterial properties.

- ❖ Potential application of silver nanoparticle like diagnostic, biomedical, optical image biological implant (heart valves) and medical applications like wound dressing contraceptive devices, surgical instruments and bone prosthesis. Used as coating for solar energy absorption and inter calculation material for electrical batteries as optical receptor as catalyst, biolabilling and as antimicrobial activity.
- ❖ Nano silver lined refrigerators, air conditioners and washing machines. Silver nanoparticles are used as antibacterial agents in the health industry food storage and textile coating. Silver nanoparticles are used as water treatment. Silver nanoparticles are used in biosenses and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection .
- ❖ Silver nanoparticles are incorporated in apparel, footwear, paints wound dressings, appliances cosmetics and plastics for their antibacterial properties.
- ❖ Silver nanoparticles are used in conductive inks and integrated into composites to enhance thermal and electrical conductivity.
- ❖ Silver nanoparticles are used to efficiently harvest light and for enhanced optical spectroscopies including Metal-Enhanced Fluorescence's(MEF) and surface-enhanced Raman Scattering.
- ❖ The size of silver nanoparticles 1 nm to 100 nm commonly used are spherical silver disease that occur on many commercially important plants like been, strawberry, peril and other crop plant. In vitro studies deal with the effect of silver nanoparticles on collie to trichom species under different concentrations and growth medium as well as the control mechanism of silver nanoparticles against colletotrichumin field trials .

1.3 Why Green Synthesis?

The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction oxidation. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed of the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost environment, environment friendly. This method need not to use of high pressure, energy, temperature and toxic chemical.

The use of plants for the preparation of nanoparticles could be more advantages, because it does not require elaborate processes such as intracellular synthesis and multiple purification step or the maintenance of microbial cell cultures. Recently, many methods have been used for the synthesis of silver nanoparticles from bio organism such as microbes, plant extract etc. In this work an inventive contribution for synthesis of silver nanoparticles using *MUNTINGIA CALABURA L(FRUITS)*, *CITRUS RETICULATA LEAVES* extract. The procedure for the development of stable silver nanoparticle is rapid and simple. Synthesized nanoparticles were characterized by various methods, such as UV-Vis spectroscopy, FT-IR, SEM, XRD and Antimicrobial activity.

1.4 PLANTS DISCRIBTION

1.4.1 a) Muntingia Calabura L(fruits)



Medicinal Uses

1. Muntingia Calabura L otherwise called kersonfruit. It is coming from cherry like flower.
2. It contain vitamin C , and high level of phosphorus, iron, calcium , protein present in this fruit.
3. To preventing the heart attack, cancer and reduce the blood pressure. It is one of the best pain reliver and antioxidant.

- To treatment of gastric ulcers, belching, heart burn vomiting senses, gout pain and arthritic. It helps in bone strengthening and cardiovascular protection.

1.4.2 (b) Citrus Reticulata Leaves



Medicinal Uses

- The fruit leaf is used as stomachic, aromatic carminative and flavouring agent. It is added in the preparation of orange tincture.
- Fruit peel oil is used in the manufacturing of perfumes, soaps and flavouring extracts, and as a drug. As a flavouring agent, it is generally recommended in non-alcoholic beverages, alcoholic beverages, ice-creams, candy, baked goods, chewing gum, gelatins and puddings, condiments, cereals, meats, syrups, etc.
- Orange squashes and concentrates, (santra squash), which are prepared from juice extracted from tight - skinned as well as from loose - skinned oranges, by use of automatic juice extractors, are available in the market as soft drink preparations and are widely used in summer season.

2.EXPERIMENTAL METHOD

2.1 Materials and Chemicals

- *Muntingia Calabura L(Fruits)*
- *Citrus Reticulata(leaves)*
- *Silver nitrate*
- *Distilled water*

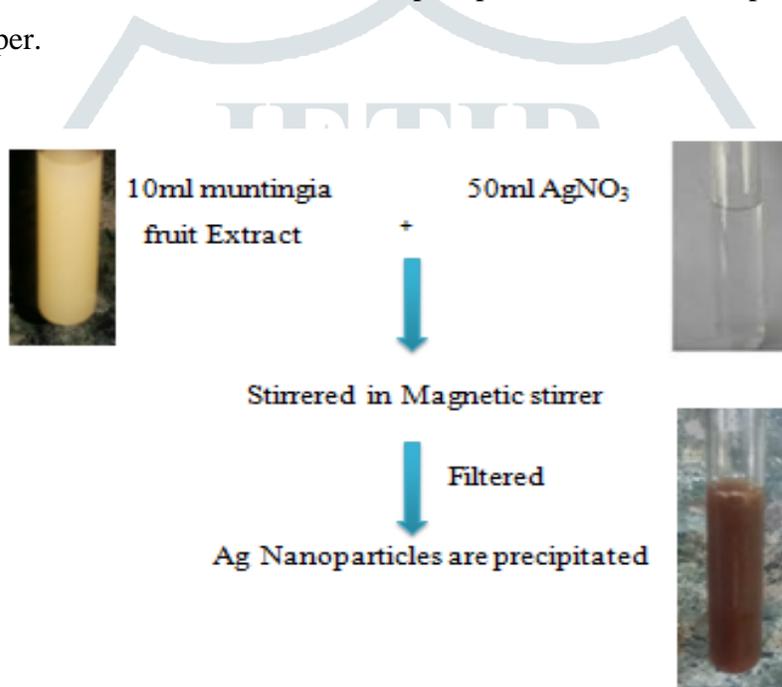
2.2 Preparation of Plant Extract

Fresh plants of *Muntingia Calabura L(Fruit)*, and *Citrus Reticulata (Leaves)*, were collected from our college campus. 25 gms of collected green leaves Flowers and Fruit were thoroughly washed with tap water and

then distilled water, cut into fine pieces, and boiled in 100 ml of distilled water, for half an hour. The aqueous extract thus obtained was filtered through whattman No.1 filter paper to obtain a clear extract. The extract was collected in clean and dried 100 ml beaker. Then the filtrates were collected and refrigerated for further experiments.

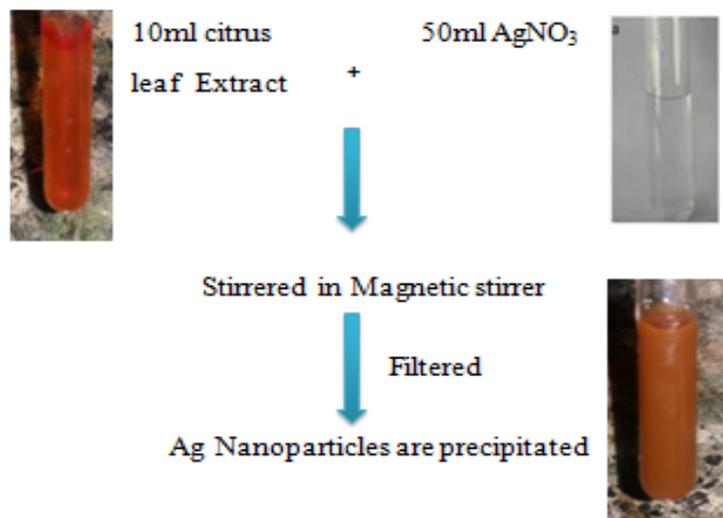
2.2.1 Synthesis of Silver Nanoparticles Using Muntingia Calabura L (fruits) Extract

Aqueous solution of silver nitrate (AgNO_3) at concentration of 0.02 mmol/ml was prepared and used for the synthesis of silver nanoparticles. The 10 ml of the above prepared fruit extract under normal concentration was taken in a test tube and 50 ml of the silver nitrate solution is added (1:5) and used for stirred method. The change of colour takes place within few minutes and the precipitate is formed. The precipitate is separated using whattman No.1 filter paper.



2.3 Synthesis of Silver Nanoparticles Using Citrus Reticulata Leaf Extract

Aqueous solution of silver nitrate (AgNO_3) at concentration of 0.02 mmol/ml was prepared and used for the synthesis of silver nanoparticles. The 10 ml of the above prepared leaf extract under normal concentration was taken in a test tube and 50 ml of the silver nitrate solution is added (1:5) and used for stirred method. The change of colour takes place within few minutes and the precipitate is formed. The precipitate is separated using whattman No.1 filter paper.



2.4 Characterization

In nanotechnology, nanoparticles synthesized either biologically or chemically must be characterized in order to understand their intrinsic properties such as size, monodispersity, aqueous stability, the net charge, adsorption to biomolecules, aggregation and flocculation in various media. This provides vital information in terms of application of these nanoparticles. For instance, it provides answers to know whether a particular nanoparticle can be used in a biological application, or else to improve their synthetic processes, or chemical functionalization.

A variety of characterization techniques are currently available some which precede the advent of nanoscience and technology and mostly drawn from material science. The development of new and integrated methods suited to probe nonmaterial is however, a continuous process. The common techniques used in the characterization of nanoparticles are ultraviolet-visible (UV) spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction studies (XRD), X-ray photoelectron spectroscopy (XPS), scanning/transmission electron microscopy (SEM/TEM), atomic force microscopy (AFM) and energy dispersion and analysis of x-rays (EDAX) .

In our studies, the silver nanoparticles were synthesized and characterized by UV-Visible spectroscopy, FT-IR, SEM and XRD spectroscopy. XRD have been obtained to confirm the nanosize of the materials. The synthesized nanoparticles were screened for antimicrobial activity.

2.4.1 UV-Visible Spectroscopy

UV-Vis spectroscopy is based on the absorbance of photons in the visible, near-UV and near-infrared regions of the electromagnetic spectrum. UV-Vis spectroscopy, as a technique of characterization, also involves the transition of electrons, and it complements fluorescence spectroscopy which deals with transitions of electrons from excited state to ground state. Generally, spectroscopy is used to identify elements and compounds for

structural elucidation of matter at the atomic and molecular levels, the most common form being ultraviolet-visible (UV/Vis) spectroscopy. Nanoparticles of silver have been extensively characterized by this technique due to their plasmonic nature and optical properties which are sensitive to size, shape, concentration and aggregation state. Although the wavelength for UV/Vis spectroscopy is within the nanoscale (i.e. $<1\mu\text{m}$), some nanomaterials have much smaller dimensions and may require other spectroscopic techniques for characterization.

2.4.2 FT-IR Spectroscopy

The Infrared region of the electromagnetic spectrum extends from the red end of the visible spectrum to the micro wave region include at the wavelength between 0.7 and 500 micrometer(or) wave number between 14,000 and 200 cm^{-1} . Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. It covers a range techniques, mostly based on absorption spectroscopy. It can be used to identify and steady chemicals. The infrared portion of the electromagnetic spectrum is usually divided into three regions: the near, mid and far-infrared. The higher energy near IR, approximately 1400-4000 cm^{-1} (0.8-2.5 μm wavelength). The mid-infrared, approximately 400-4000 cm^{-1} (2.5-25 μm) may be used to study the fundamental vibrations and associated rotational-vibrations structure.

2.4.3 X-Ray Diffraction Pattern

The X-ray diffraction analysis investigates structure through the use of diffraction. The method of X-ray diffraction analysis are used to study, for example, metals, alloys, minerals, inorganic and organic compounds. Polymers, liquids, gases and the molecule of proteins and nuclei acids. The X-ray diffraction analysis has been used to establish the atomic structure is crystalline substance. The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang. Samples were air dried, powdered and used for XRD analysis. X-ray Diffraction patterns were recorded in the scanning mode on Miniflex 600 Desktop (Fist support) analytical instrument operated at 40 KV current of 30 mA.

For determination of crystalline size, Scherrer analysis of XRD is commonly used. This technique relies on the broadening of diffraction peaks due to the finite number of diffracting planes. Because other factors, such as strain, can broaden XRD peaks, Scherrer analysis generally provides a lower limit on mean crystalline size. XRD measurements are performed using a Philips diffractometer of 'X' pert company with mono chromatized ($\lambda=1.54060 \text{ \AA}$) radiation. Particle size is determined from the width of XRD peaks using Scherer's formula,

$$D = K/\text{Cos}\theta$$

$$D (\text{nm}) = 0.9\lambda/\beta\text{cos}\theta$$

Where D is the average particle size in nm, λ is the wave length of the X-ray, θ is the brass diffraction angle in the degrees, β is the full of the peak at half height in radius.

2.4.4 Scanning Electron Microscopy

SEM is recorded by JEOL model 6390 computer- controlled microscope. The image obtained by SEM of the sample for Ag show quasi spherical like nanoparticles. The Ag nanoparticles have been distributed well within the range 27 of $\sim 100\text{nm}$. The most important in scanning electron microscope is the use of electrons. Much logically flows from this, such as that a vacuum is needs to generate an electron beam, that electrons are used for imaging, and that we need to understand the interactions of electron beam with the sample in order to interpret the images. The use of electrons also impacts on image resolution and image color, and also explains the 3D nature of the micrographs (photos). The second most important concept is that we are looking only at the surface of the sample, and penetrating only on a small way into the sample with the electron beam shows that the SEM instrument images are displayed as monochromatic gray scale digital images in which each pixel carries only intensity information in shade of gray varying form be black at weakest intensity to white at the stronger. Sometimes these gray scale images be post-produced to display false color i.e. colorized gray scale.

2.4.5 Antimicrobial Activity

Preparation of Plates

The medium is prepared and sterilized as directed by the manufactured defibrinated blood may be necessary for tests on fastidious organisms, in which case the medium should be allowed to cool to 50°C before 7% of blood is added. Human blood is not recommended as it may contain antimicrobial substances. The medium should be poured into Petri dishes on a flat horizontal surface to a depth of 4mm (25ml in an 85mm circular dish 60ml in a 135mm circular dish) poured plates are stored $+4^{\circ}\text{C}$ and used within one week of preparation.

Before inoculation plates should be dried with lids a jar so that there are no droplets of moisture on the agar surface. The time to achieve this depends on the drying conditions.

The p^{H} of the medium should be checked at the time of preparation and should be 7.2 -7.4.

Preparation of Inoculums

At least four morphologically similar colonies from an agar medium are touched with a wire loop and the growth is transferred to a test tube containing 1.5 ml of sterile suitable broth. The tubes are incubated for 2 hours at 35°C to 37°C to produce a bacterial suspension of moderate turbidity. The density of the suspension is standardized by dilution with sterile saline or broth to a density equivalent to the barium sulphate standard, 0.5 McFarland units. Before use the standard should be shaken vigorously.

Inoculation

Plates are inoculated within 15 minutes of preparation of the suspension so that the density does not change. A sterile cotton - cool swab is dipped into the suspension and surplus removed by rotation of the swab

against the side of the tube above the fluid level. The medium is inoculated by even streaking of the swab over the entire surface of the plate in three directions.

Antibiotics discs

After the inoculum has dried, single discs are applied with forceps, a sharp needle or a dispenser and pressed gently to ensure even contact with the medium. When fastidious organisms are to be tested, touch multiple colonies with a loop and cross streak the appropriate plate for uniform distribution.

Not more than six discs can be accommodated on an 85 mm circular plate and twelve are easily accommodated on a 135mm circular plate.

Disc should be stored at +4⁰ C in sealed containers with a desiccant and should be allowed to come to room temperature before the containers are opened. Disc should be used before the expiry date on the label. If antimicrobial solution prepared in the laboratory are being used proceed.

- Pick up a 2 mm loopful of the standard antibiotic solution and lower carefully onto a paper disc which, when moistened will adhere to the loop.
- Place the moistened disc on the surface of inoculated plate in the appropriately labeled segment.

NOTE : Take care to avoid inadvertent “contamination” of other discs in the Petri dish with a antibiotic solution.

- Repeat for each antimicrobial agent to be used, placing the impregnated discs in their respectively labeled segments.

Incubation

Plates are incubated for 16 -18 hours at 35 to 37⁰ C aerobically or in CO₂ atmosphere for fastidious organisms.

Reading of zones of inhibition

The diameters of zones are measured to the nearest millimeter with vernier calipers or a thin transparent millimeter scale. The point of abrupt diminution of growth, which in most cases corresponds with the point of complete inhibition of growth is taken as the zone edge. In some batches of media, organisms may show a film of growth within the susceptible zone which may be ignored. Similar findings may be seen with swarming proteus Spp.

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

The plant extracts were used to produce silver nanoparticles, the reduction of silver ions silver nanoparticles occurred after mixing silver nitrate with different plant and species extract, followed color change of solutions due to reduction of silver ion, which may be indicating of formation silver nanoparticles were

characterized by UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), X-Ray diffraction (XRD), Infrared (IR) was used to characterize the crystal structure, morphologies, impurities and optical properties of silver nanostructure. The synthesized nanoparticles are screened against Antimicrobial Activity.

3.2 Synthesis of Plant Extract

Fresh plants were collected, 25 gms of collected green plants were thoroughly washed with tap water and then distilled water, cut into fine pieces, and boiled in 100 ml of distilled water, for half an hour. The aqueous extract thus obtained was filtered. The extract were characterized by UV-Visible spectroscopy, X-Ray Diffraction (XRD), Infrared (IR) Scanning Electron Microscopy (SEM) was used to characterized the crystal structure, morphologies, impurities and optical properties of extract.

3.3 UV-Visible Spectroscopy

The bio reduction of silver in aqueous solution was monitored by periodic sampling of aliquots of the mixture and subsequently measuring UV-Visible spectra. UV-Visible spectral analysis was done by using shiatsu UV-1800 double beam spectrophotometer. The absorption peaks are measured in the rang 200-800nm. The UV-Visible spectrum is recorded in acetone solvent by shimadzu 1800 UV Double beam spectrophotometer. UV-Visible spectrum has been widely used to characterize the semiconductor nanoparticles. As the particles size decreases absorption wave length will be shifted to shorter wave length and the band gap increases for the nano sized particles. This is the quantum confinement effect of semiconductor nano particles. UV-Visible spectroscopy analysis showed that the wave length of silver nanoparticle synthesized using Muntingia Calabura L, and Citrus Reticulata Leaf extract centered at **400-500nm** due to the excitation of surface.

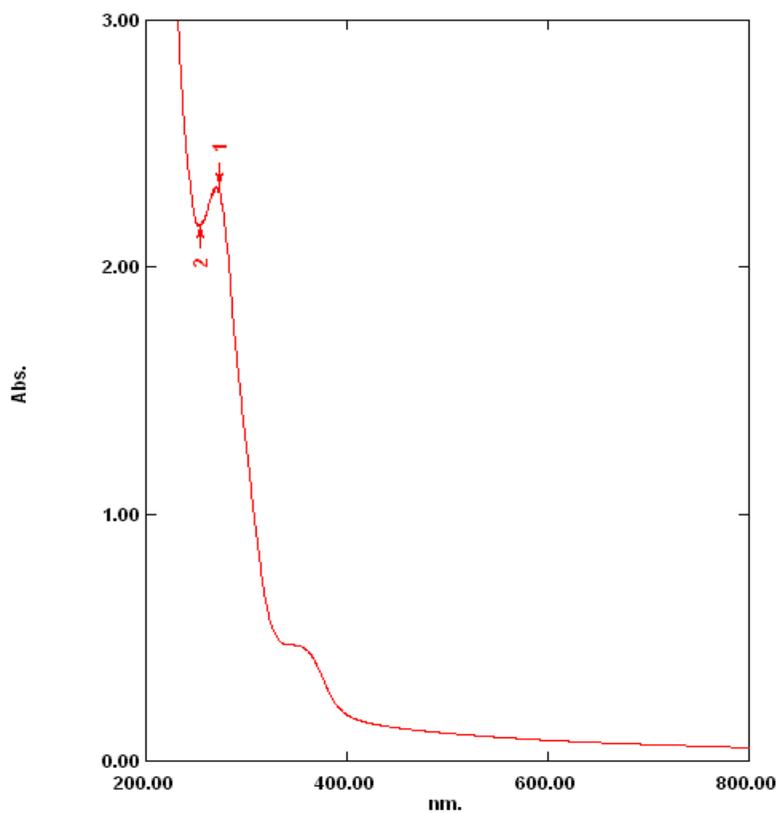


Fig 3.1 (b:1) UV Spectrum of Muntingia Calabura L Extrat

Wavelength nm	Absorption
272.50	2.34
253.50	2.16

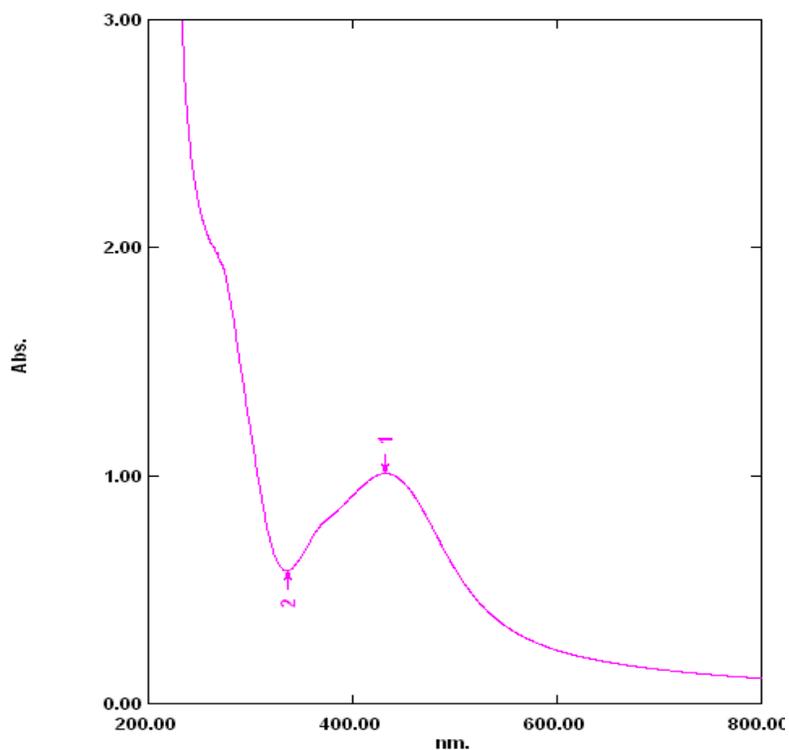


Fig 3.2 (b:2) UV Spectrum of Silver Nanoparticle from Muntingia Calabura L

Wavelength nm	Absorption
431.50	1.01
336.00	0.58

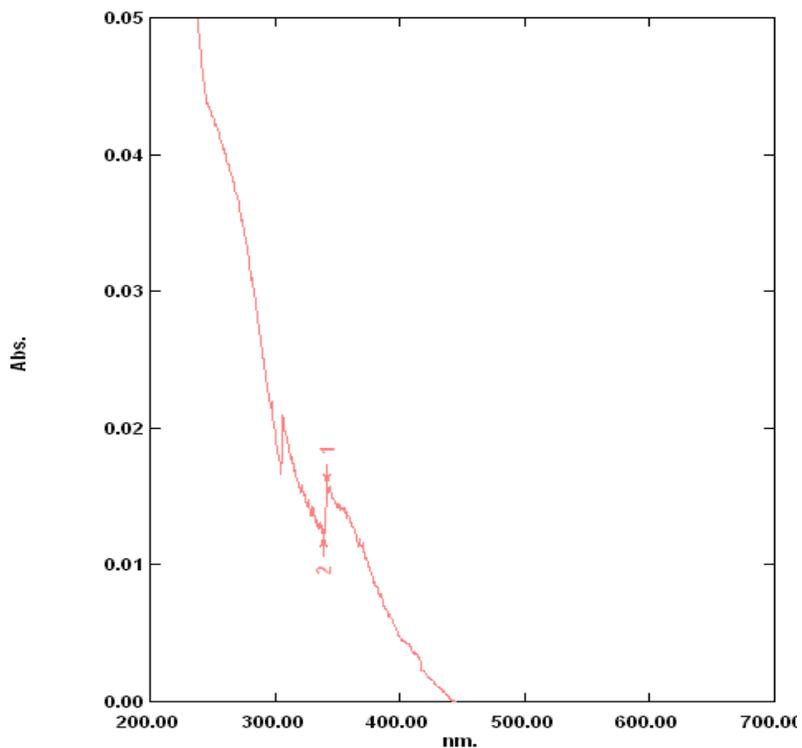


Fig 3.3 (c:1) UV Spectrum of Citrus Reticulata Leaves Extract

Wavelength nm	Absorption
342.00	0.02
339.00	0.01

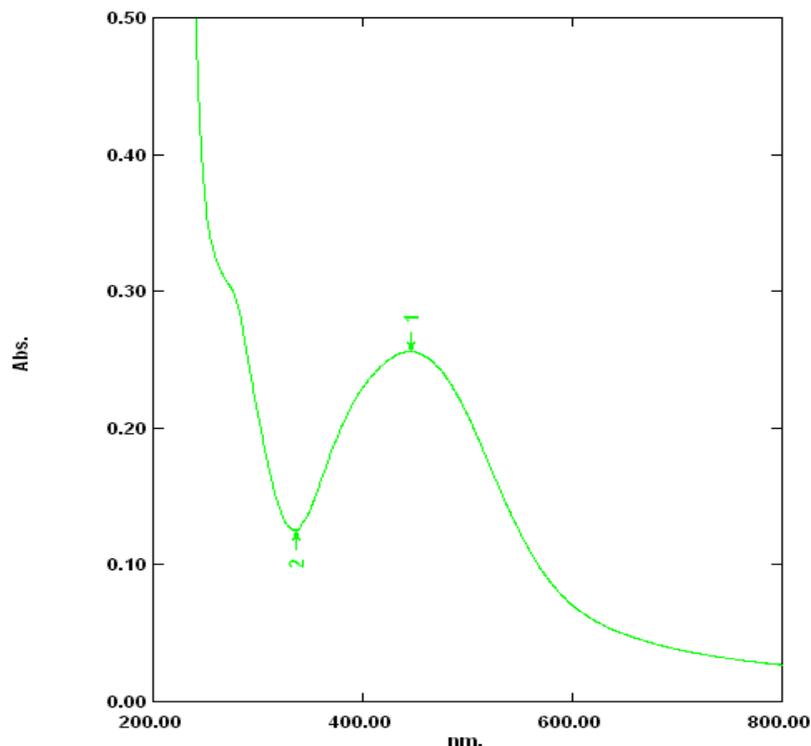


Fig 3.4 (c:2) UV Spectrum of Silver Nanoparticle from Citrus Reticulata Leaves

Wavelength nm	Absorption
445.50	0.26
336.00	0.13

In this spectra for silver nanoparticle is observed at **445 nm**. This indicates the absorption shift towards the shorter wavelength, because of the particle size reduction. From these spectra, it is evident that resultant nanoparticles were embedded in silica matrix and exhibited the significant blue shift. This is an indicating of strong quantum confinement. The bulk value for Ag is at 400-500nm.

3.4 FT-IR Analysis

FT-IR analysis is utilizable for characterizing the surface chemistry of nanoparticles. Organic functional groups like OH, C=O linked to the surface of nanoparticles are found by FT IR. The FT-IR spectrum is recorded in acetone solvent by shimadzu 1800 UV double beam spectrophotometer. For FT-IR measurements, Silver nanoparticle solution was centrifuged at 20,000 rpm for 20 minutes the pellet was washed three times with 20 ml

of de-ionized water. The samples were dried and analyzed on IR-Prestige-21 [SHIMADZU] operating at a resolution of 2 cm^{-1} . Further the FTIR analysis of the leaves, fruits and flower extracts mediated silver nanoparticles was performed. The spectra gave maximum peaks at 1633cm^{-1} , 3790cm^{-1} , 1031cm^{-1} , 1444cm^{-1} , 2954cm^{-1} which indicate the presence of alcohols/phenols, aldehydes, carbonyls, alkanes, alkenes and aliphatic amines, Nitro group,.

Infrared spectroscopy (IR) is the spectroscopy that deals with the Infrared region of the electromagnetic spectrum that is light with a longer wave length and lower frequency than visible light. It covers a range of techniques, Mostly based on the absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. A common laboratory instrument that uses this technique is a Fourier Transform Infrared *(FT-IR) spectrophotometer.

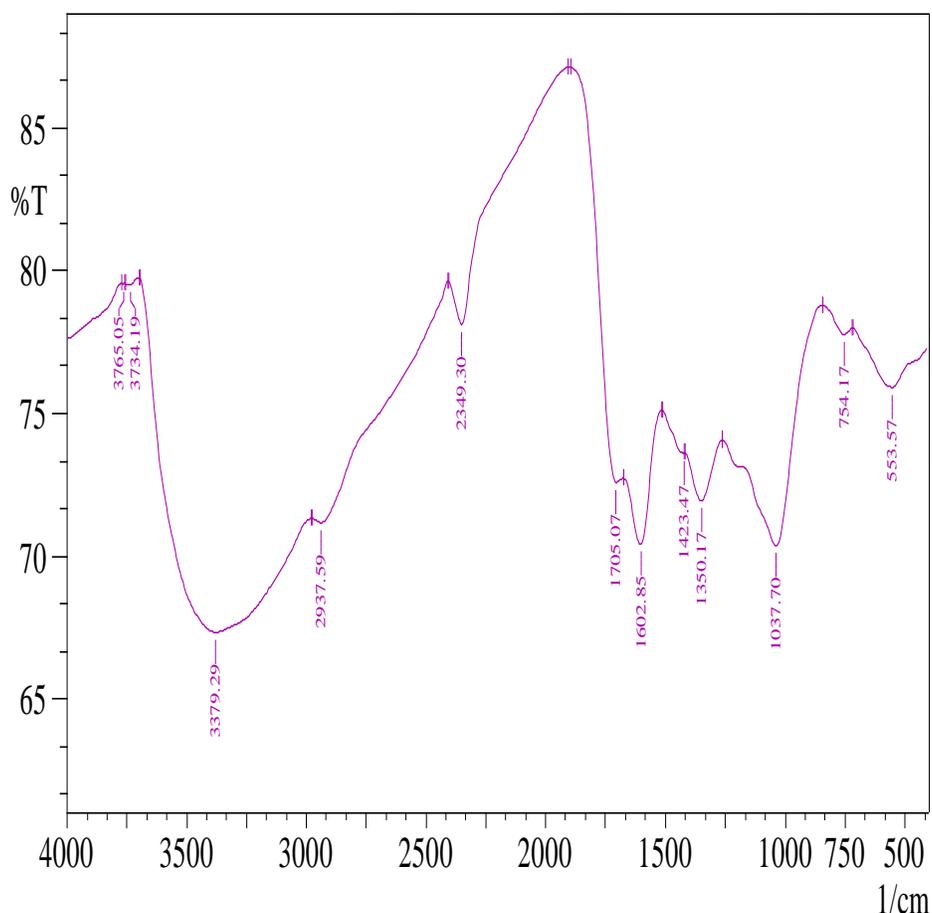


Fig 3.4 .1(b:1) FT - IR Spectrum of Muntingia Calabura L

Frequency	$\nu(\text{C}=\text{C})$ Stretch	$\nu(\text{O}-\text{H})$ Stretch	$\nu(\text{C}-\text{O})$ Stretch	$\nu(\text{N}-\text{H})$ Stretch	$\nu(\text{C}-\text{H})$ Stretch	$\nu(\text{C}-\text{O}-\text{C})$ Stretch	$\nu(\text{N}-\text{O}_2)$ Stretch
Muntingia CalaburaM	1602cm ⁻¹	3765 cm ⁻¹	1037 cm ⁻¹	3379 cm ⁻¹	2937 cm ⁻¹	1037 cm ⁻¹	1350 cm ⁻¹

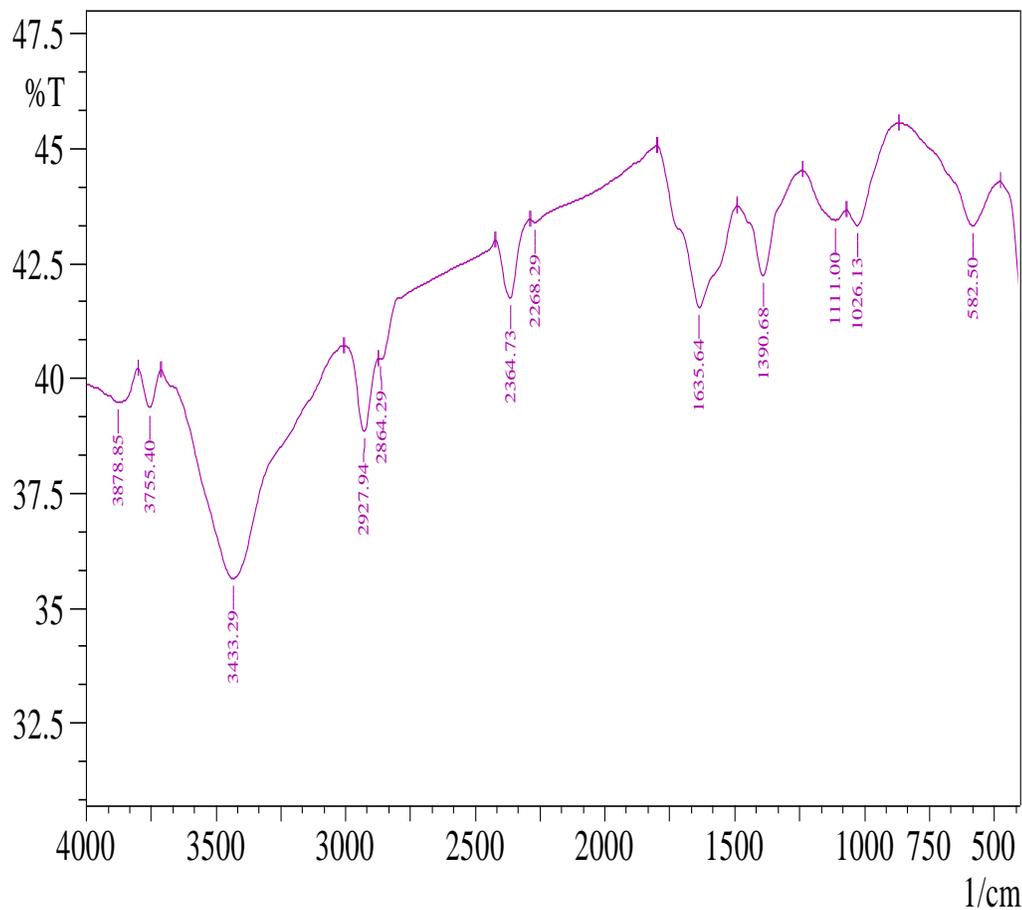


Fig 3.4.2 (b:2)FT – IR Spectrum of silver Nanoparticle from Muntingia Calabura

Frequency ν	$\nu(\text{C}=\text{C})$ Stretch	$\nu(\text{O}-\text{H})$ Stretch	$\nu(\text{C}-\text{O})$ Stretch	$\nu(\text{N}=\text{C}=\text{O})$ Stretch	$\nu(\text{C}-\text{H})$ Stretch	$\nu(\text{C}-\text{O}-\text{C})$ Stretch	$\nu(\text{N}-\text{O}_2)$ Stretch
Muntingia Calabura L	1635 cm ⁻¹	2364 cm ⁻¹	1026 cm ⁻¹	2268 cm ⁻¹	2927 cm ⁻¹	1026 cm ⁻¹	1390 cm ⁻¹

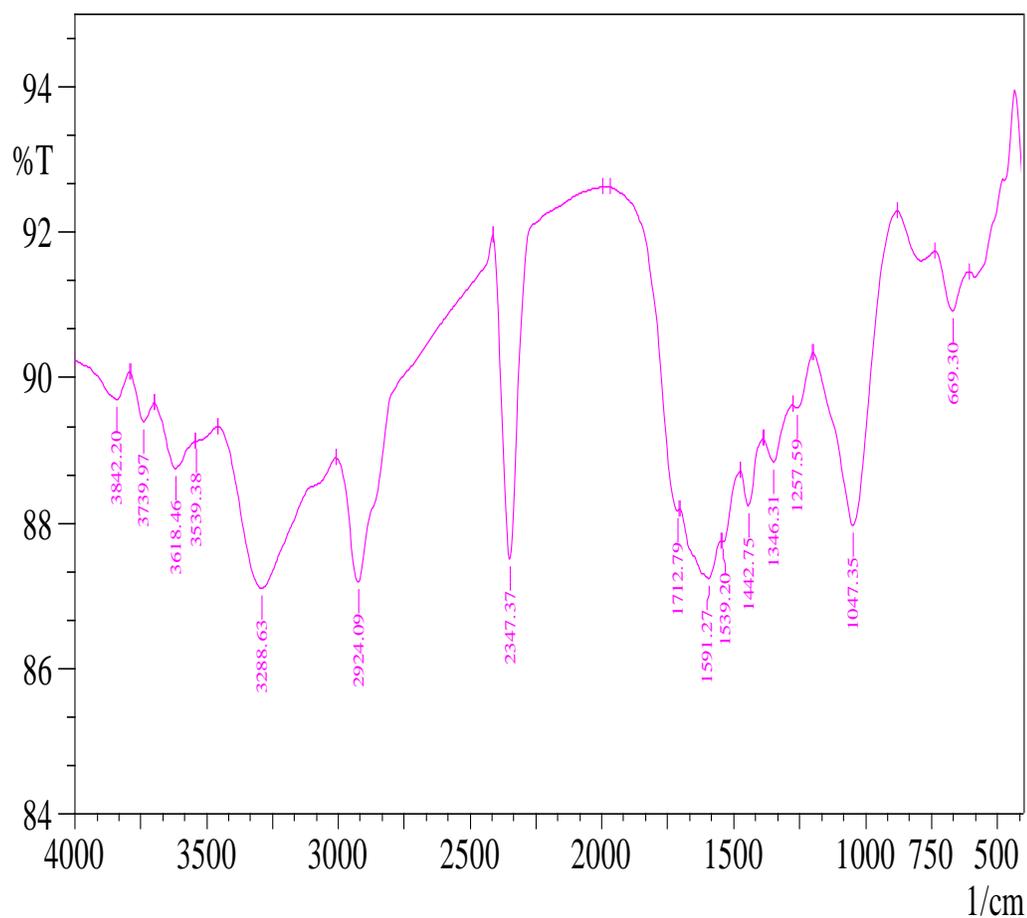


Fig 3.4.3 (c:1)FT - IR Spectrum of Citrus Reticulata Leaves Extract

Frequency ν	$\nu(\text{C}=\text{C})$ Stretch	$\nu(\text{O}-\text{H})$ Stretch	$\nu(\text{C}-\text{O})$ Stretch	$\nu(\text{C}=\text{O})$ Stretch	$\nu(\text{C}-\text{H})$ Stretch	$\nu(\text{C}-\text{O}-\text{C})$ Stretch	$\nu(\text{N}-\text{O}_2)$ Stretch
Citrus Reticulata leaves	1591cm ⁻¹	3618cm ⁻¹	1047cm ⁻¹	1721cm ⁻¹	2924cm ⁻¹	1257cm ⁻¹	1346 cm ⁻¹

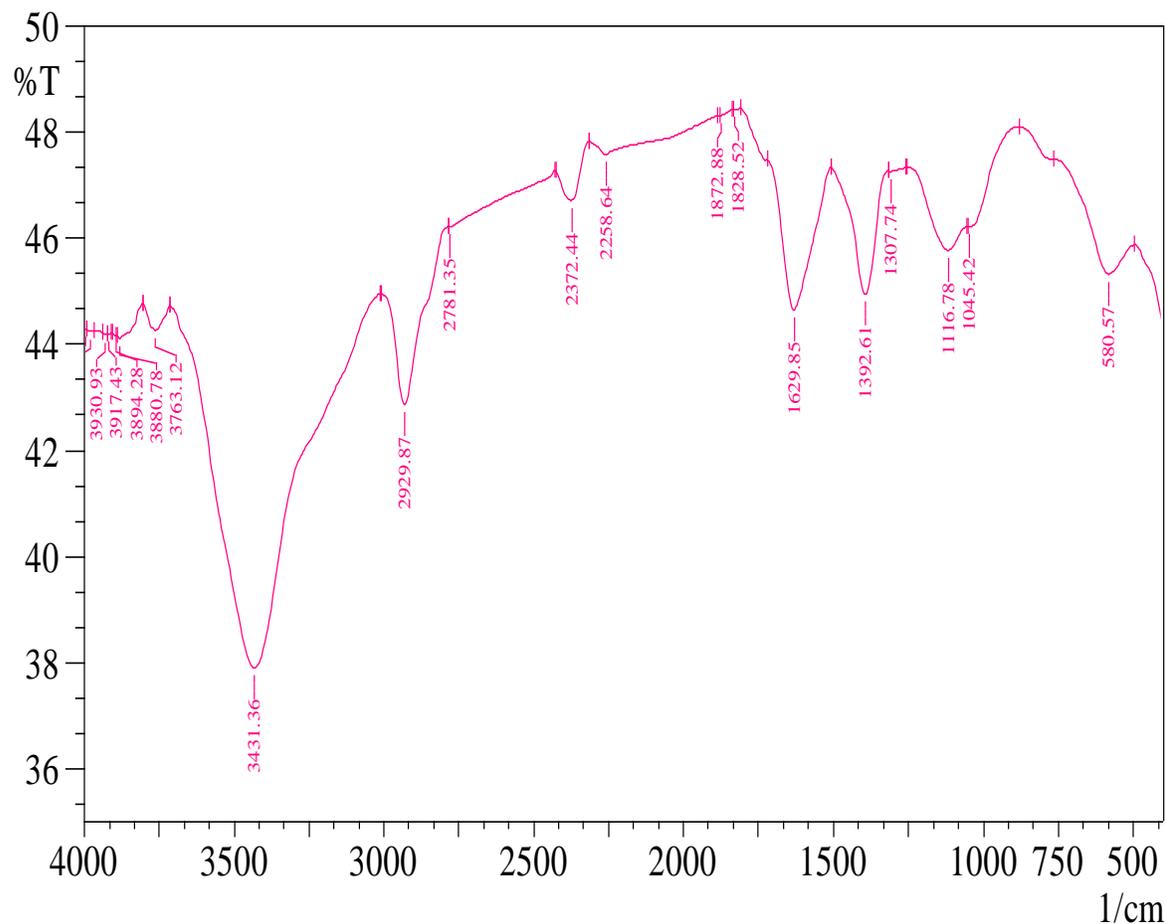


Fig 3.4.4 (c:2)FT - IR Spectrum of Silver Nanoparticle from Citrus Reticulata Leaves

Frequency ν	$\nu(\text{C}=\text{C})$ Stretch	$\nu(\text{O}-\text{H})$ Stretch	$\nu(\text{C}-\text{O})$ Stretch	$\nu(\text{N}-\text{H})$ Stretch	$\nu(\text{C}-\text{H})$ Stretch	$\nu(\text{C}-\text{O}-\text{C})$ Stretch	$\nu(\text{N}-\text{O}_2)$ Stretch
Citrus Reticulata leaves	1629 cm^{-1}	2372 cm^{-1}	1045 cm^{-1}	3431 cm^{-1}	2781 cm^{-1}	1116 cm^{-1}	1392 cm^{-1}

3.5 X-Ray Diffraction Studies

The silver nanoparticle solution obtained was purified by repeated centrifugation at 5000 rpm for 20 minutes followed by redispersion of the pellet of silver nanoparticles into 1ml of deionized water. After freeze drying of the purified silver nanoparticles, the structure and composition were analyzed by XRD. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the scherer formula

The XRD spectrum is recorded by X-ray diffract meter with Cu radiation at 25°C. The average particle size is determined using **Debye-scherrer's equation**

$$D = k \lambda / \beta \cos \theta$$

$$D = 0.94 \lambda / \beta \cos \theta$$

Where

- D is the average crystallite domain size perpendicular to the reflecting planes,
- λ Is the X-ray wavelength,
- β Is the full width a half maximum (FWHM), and
- θ is the diffraction angle.
- To eliminate the addition instrumental broadening the FWHM was corrected, using the FWHM from a large grained SI sample.
- B corrected = (FWHM² sample - FWHM²si)

3.5.1 Determination of Structural Parameter

From the XRD profiles, the inter planar spacing d_{hkl} was calculated using Bragg's relation,

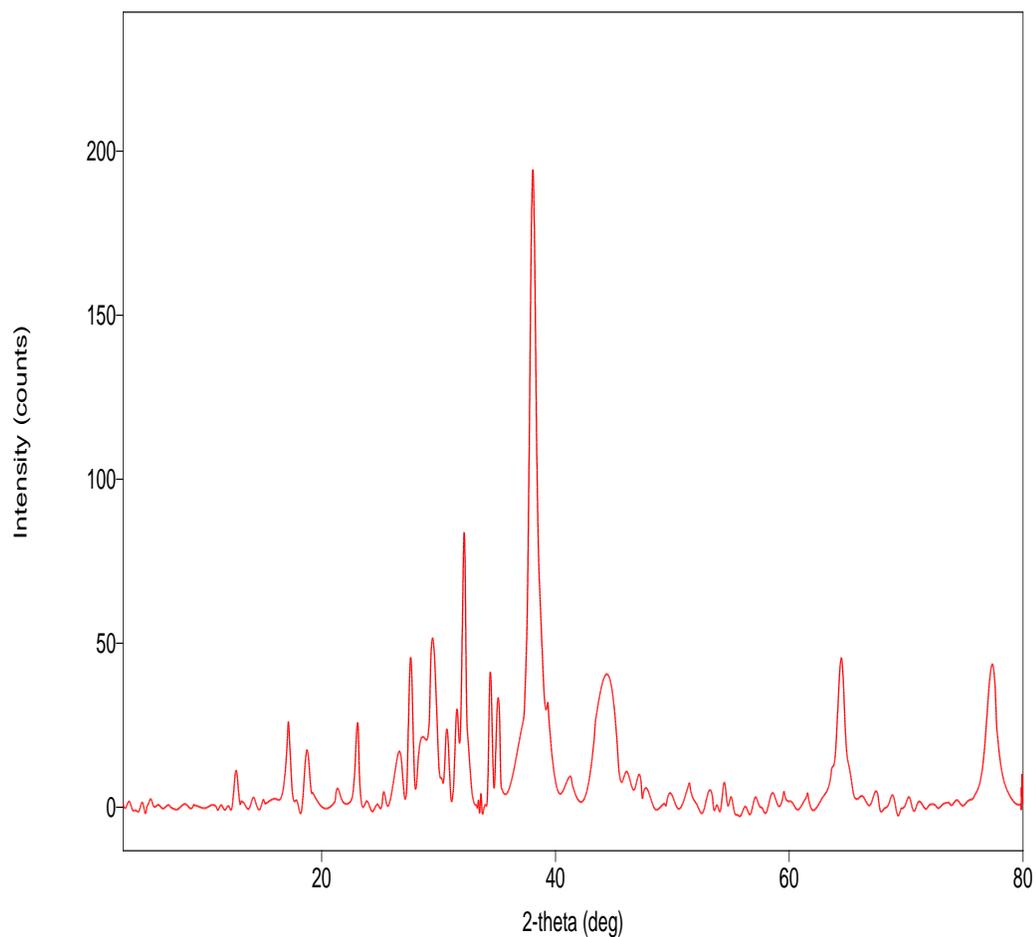
$$d_{hkl} = n\lambda / 2 \sin \theta$$

The crystalline size (D) was calculated using the formula from the full width at half maximum (FWHM).

$$D = k \lambda / \beta \cos \theta$$

Where,

- The constant K is the shape factor 0.94,
- λ is the wavelength of the X-ray (1.5406 Å for Cu),
- θ is the the Bragg's angle and ' β ' is the **FWHM**.



3.5 .2 (b:1) XRD Spectrum of Muntingia Calabura L Extract

$$2\theta = 30.4808$$

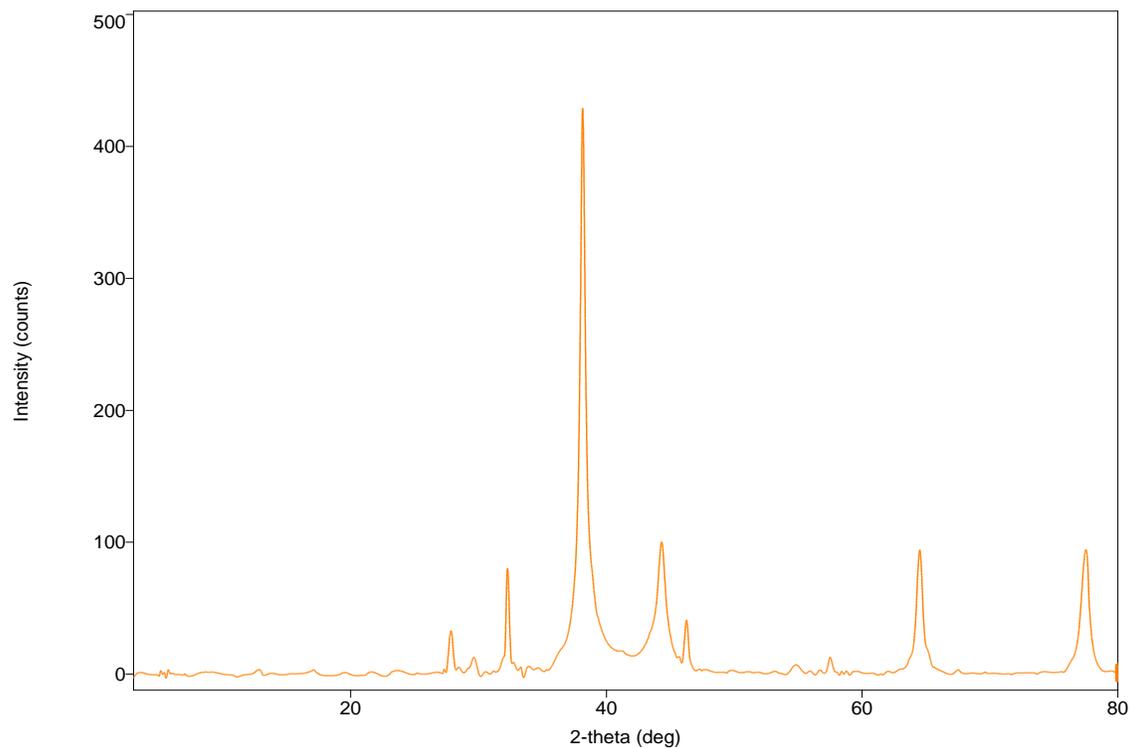
$$\theta = 30.4808/2$$

$$\cos\theta = 0.9648$$

$$D = 0.94 \times 1.5406 \sqrt{2.2884 \times 0.9648}$$

$$D = 1.448164 / 2.207$$

$$D = 0.6558 \text{ nm}$$



3.5.3 (b:2) XRD Spectrum of Ag Nano from Muntingia Calabura

$$2\theta = 44.37$$

$$\theta = 44.3729 / 2$$

$$\theta = 22.1864$$

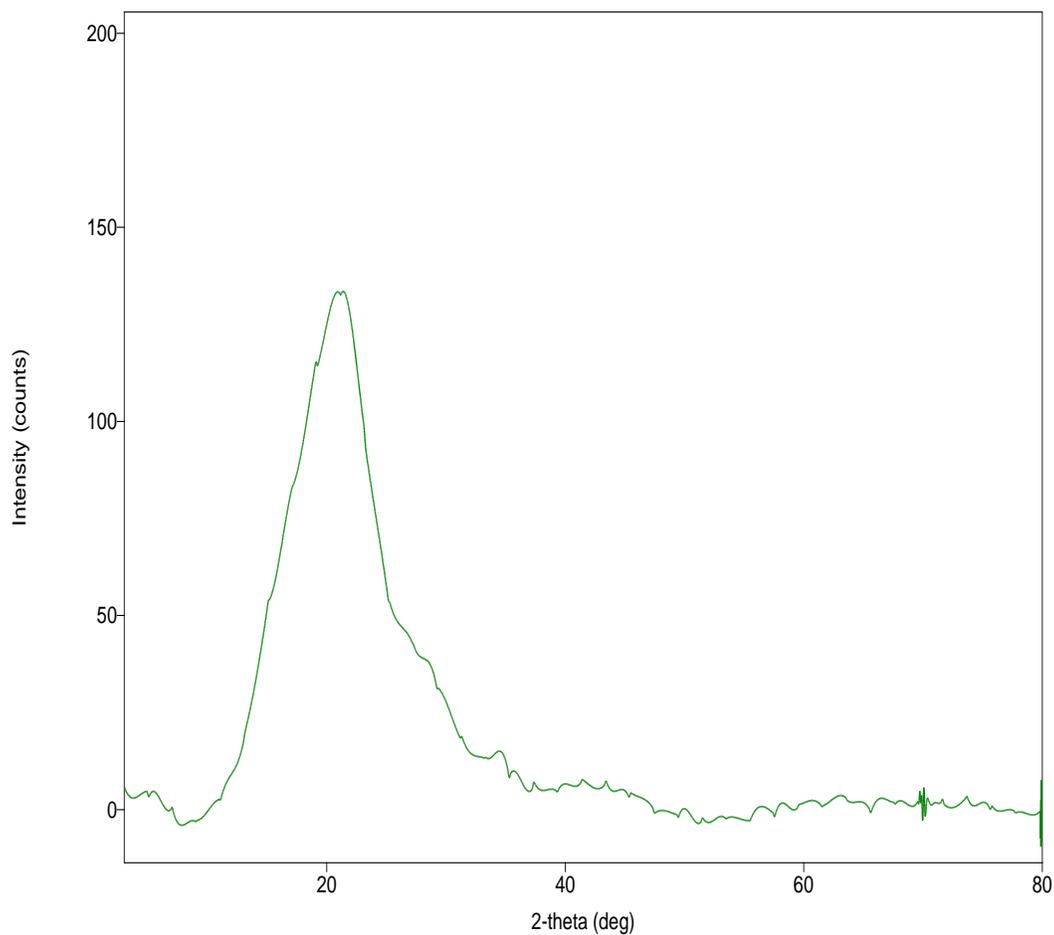
$$\cos\theta = 0.9259$$

$$D = 0.94 \times 1.5406 / 0.7302 \times 0.9259$$

$$D = 1.448164 / 0.6761$$

$$D = 2.1418\text{nm}$$

This XRD value confirms that the synthesized particles were nanomeric in the size. The size of the silver nanoparticles thus estimated was found to be 2.1418nm.



3.5.4 (c:1) XRD Spectrum of Citrus Reticulata Leaves

$$2\theta = 21.2161$$

$$\theta = 21.2161/2$$

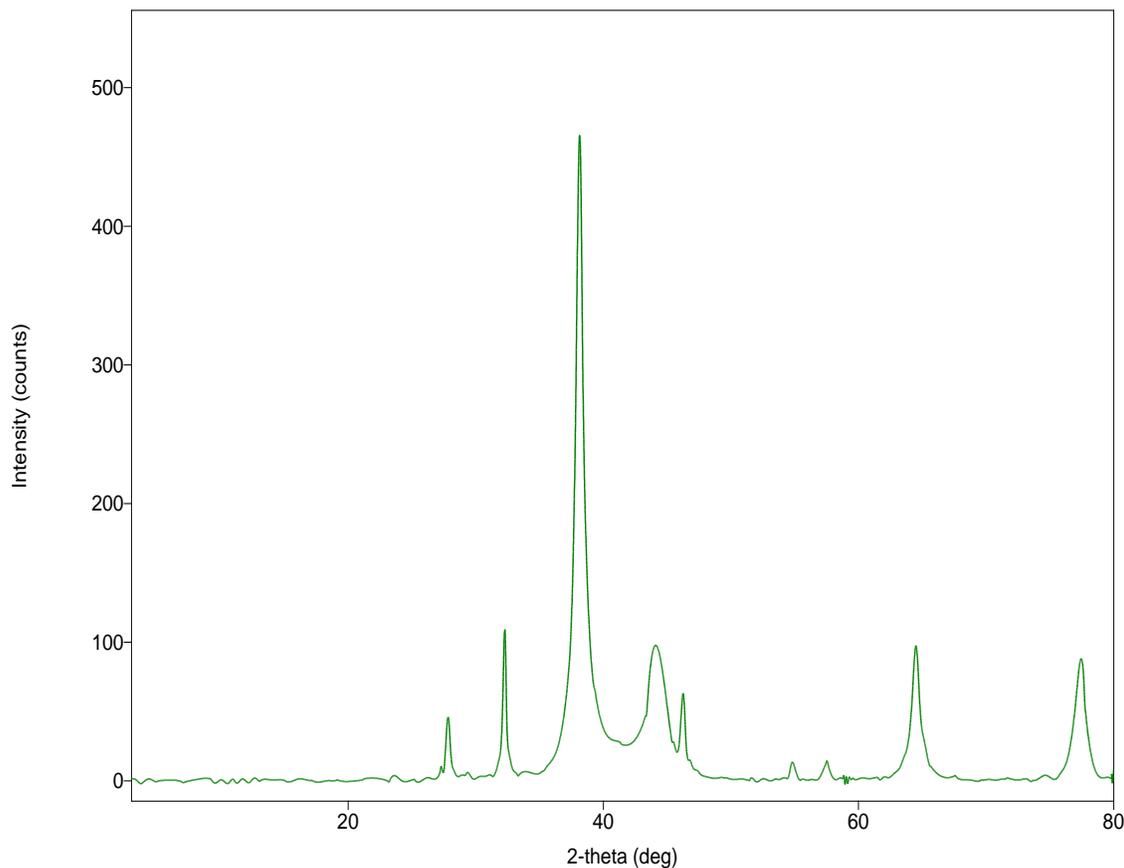
$$\theta = 10.6080$$

$$\cos\theta = 0.9829$$

$$D = 0.94 \times 1.5406 / 8.4363 \times 0.9829$$

$$D = 1.448164 / 10.4266$$

$$D = 0.1388 \text{ nm}$$



3.5.5 (c:2) XRD Spectrum of Ag Nano from Citrus Reticulata Leaves

$$2\theta = 38.0719$$

$$\theta = 38.0719 / 2$$

$$\theta = 19.0359$$

$$\cos\theta = 0.9453$$

$$D = 0.94 \times 1.5406 / 0.71750 \times 0.9453$$

$$D = 1.448164 / 0.67825$$

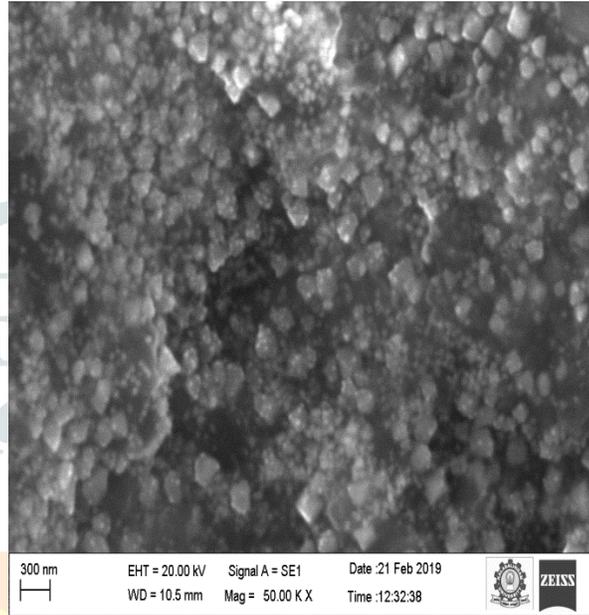
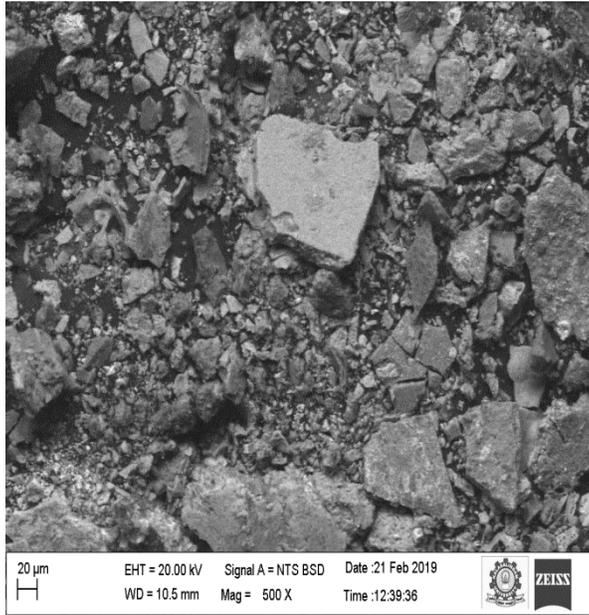
$$D = 2.1351 \text{ nm}$$

This XRD value confirms that the synthesized particles were nanomeric in the size. The size of the silver nanoparticles thus estimated was found to be 2.1351 nm.

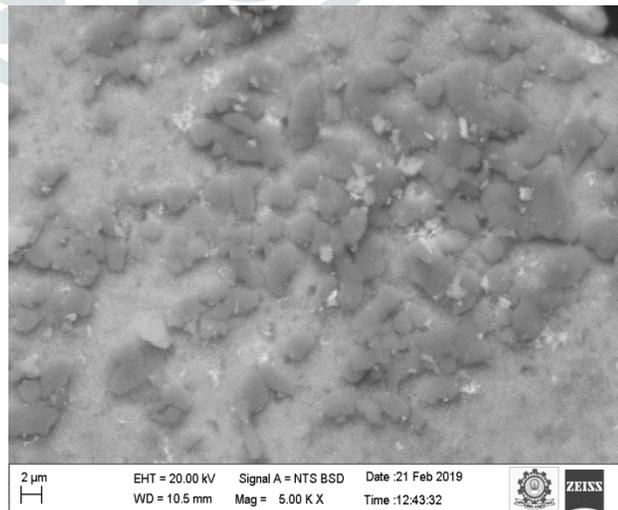
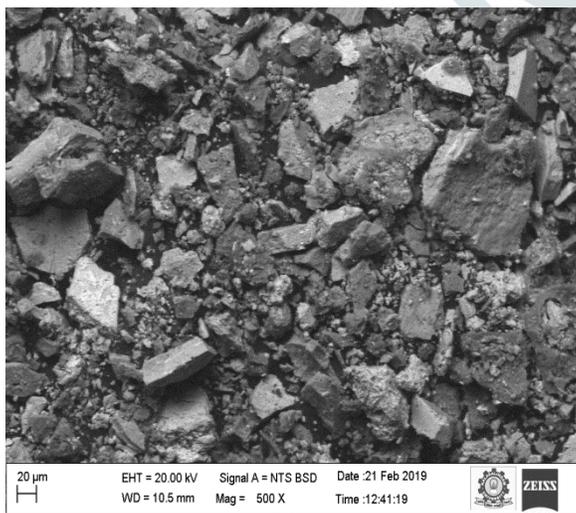
3.6 Scanning Electron Microscopy

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electron interact with electrons in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and

composition. The electron beam's position is combined with the detected signal to produce an image[5]. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum, low vacuum and environmental SEM specimens can be observed in wet condition. Size, shape and distribution of green synthesized silver nanoparticles were characterized by scanning electron microscope[6]. The particle morphology of the silver nanoparticles fabricated using *Muntingia Calabura L*, and *Citrus Reticulata* leaves.



SHAPE OF SILVER NANOPARTICLE FROM MUNTINGIA CALABURA L

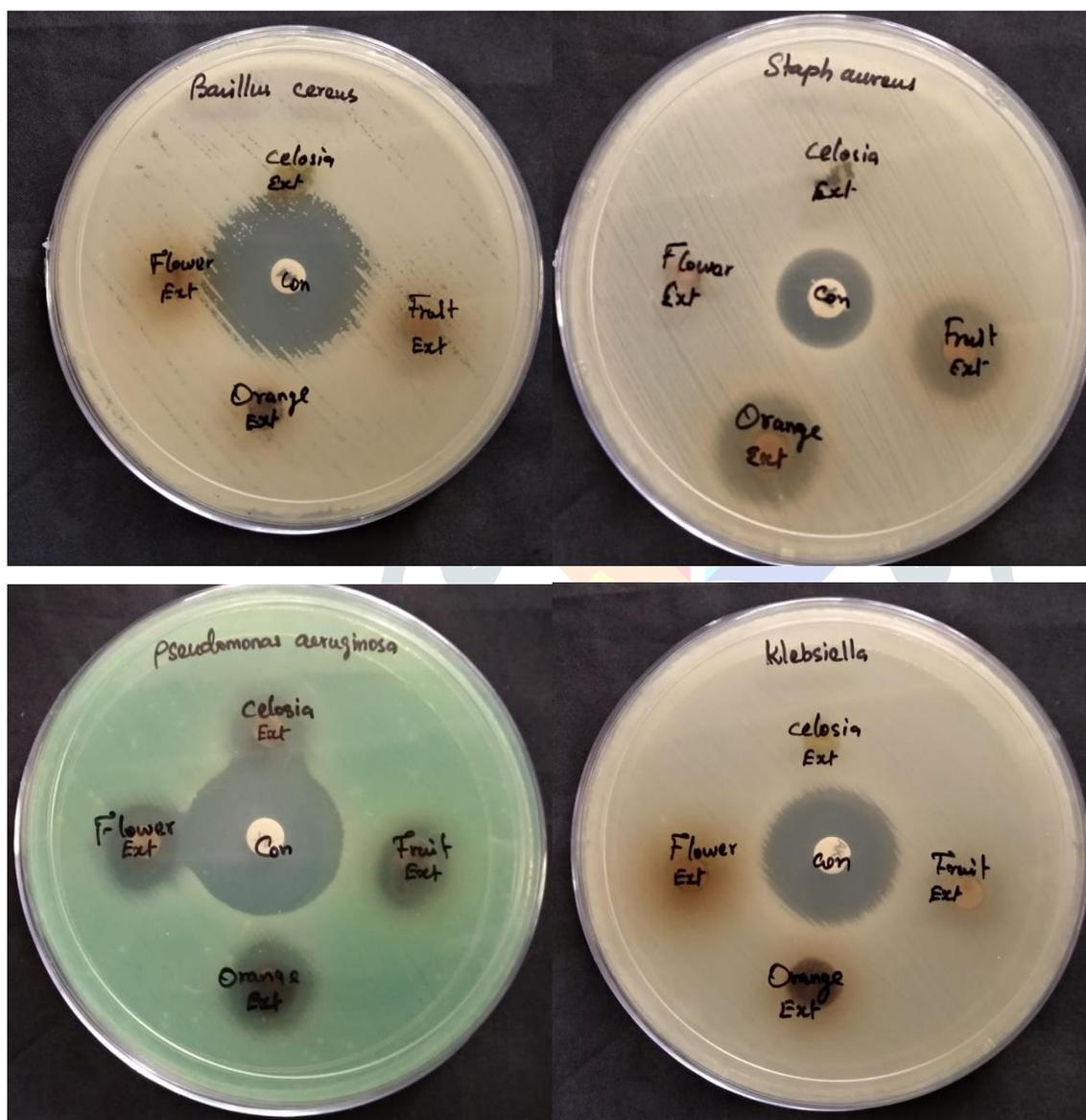


SHAPE OF SILVER NANOPARTICLE FROM CITRUS RETICULATA

3.7 Antimicrobial Activity

Silver is powerful and natural antibiotic and antibacterial agent. Silver nitrate has long been considered as powerful and natural antibacterial agents. Synthesized silver nano particle were tested for antimicrobial activity against pathogenic bacteria organisms. The antimicrobial assay was done on human pathogenic *Klebsiella*, *Pseudomonas aeruginosa* and Staph aureus, Bacillus cereus by The Kirby - Bauer method.

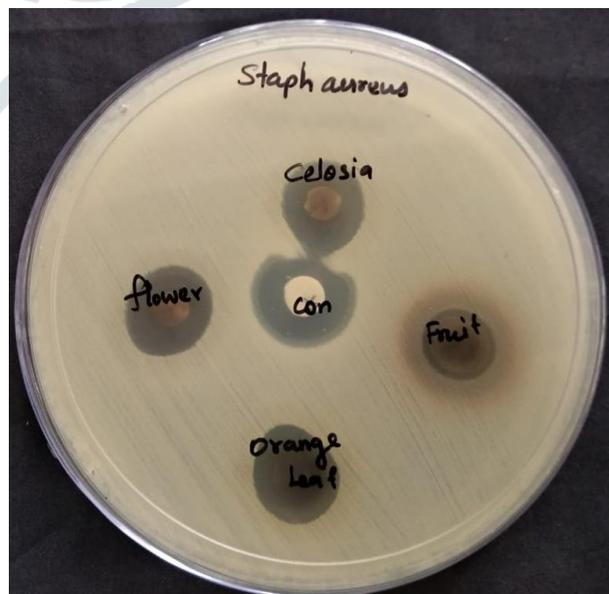
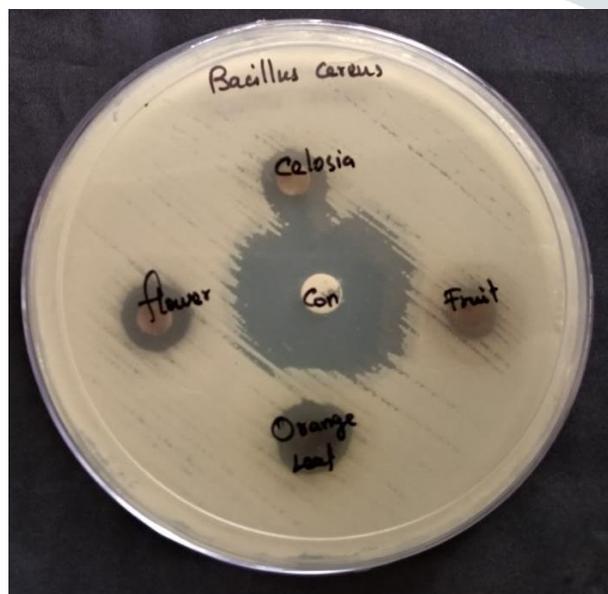
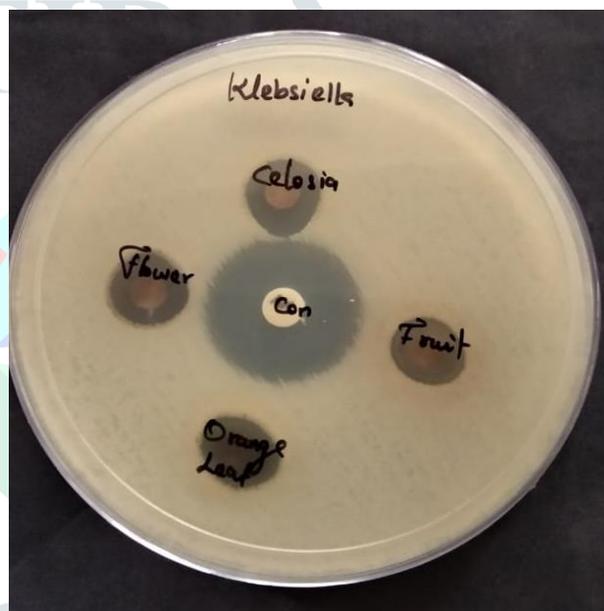
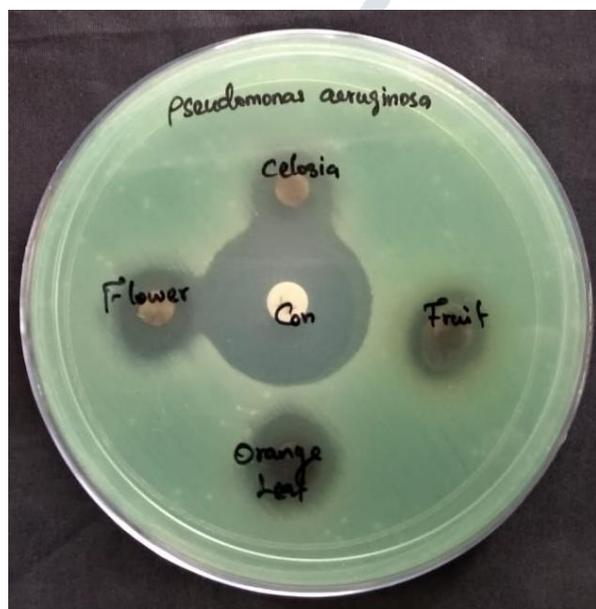
Each zone size is interpreted according to the organism by reference to the tables 5.7.1 and 5.7.2.



3.7.1

BACTERIA	Muntingia Calabura L	Citrus Reticulata	Control
Klebsiella(mm)	-	-	24
Pseudomonas aeruginosa(mm)	13	15	29
Staph aureus(mm)	16	17	17
Bacillus cereus (mm)	-	-	30

ANTIMICROBIAL ACTIVITY FOR PLANT EXTRACTS



BACTERIA	Muntingia Calabura L	Citrus Reticulata	Control
Klebsiella(mm)	12	13	24
Pseudomonas Aeruginosa(mm)	16	16	29
Staph Aureus (mm)	12	16	17
Bacillus Cereus (mm)	8	12	30

3.7.2 ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES FORM PLANT EXTRACT

4.CONCLUSION

A Critical need in the field of nanotechnology is the development of reliable and eco-friendly processes for the synthesis of metallic nanoparticles. Here, we synthesized a simple biological and low-cost approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with a bio reduction method using Muntingia Calabura L(fruit),Citrus Reticulata (leaves), aqueous extract as the reducing agent. Biologically synthesized silver nanoparticles could be of immense use in pharmaceutical field for their efficient antibacterial and antimicrobial properties. The plant extracts and the synthesized silver nanoparticles were characterized by UV-Visible spectroscopy ,FT-IR spectroscopy ,XRD, SEM and Antimicrobial activities of synthesized nanoparticle were evaluated. The result confirmed the reduction of silver nitrate to silver nanoparticle with high stability and without any impurity. The formation of silver nanoparticles was confirmed by color changes from pale yellow to dark brown color (Muntingia Calabura L fruit) and orange to pale brown (Citrus Reticulata leaf) it was confirmed by UV-Visible spectra showed a broad peak located at 250-350 nm for plant extracts. It changes to 400-500 nm after adding silver nitrate solution. FT-IR revealed that the resultant confirmed by alkynes , alkanes , alkenes, alkylhalides , dialkyl ethers, aliphatic Nitro groups, amine groups, phenol groups are present in both plant extracts and the surface of the nanoparticles. X-ray diffraction pattern (XRD) revealed that the resultant nanoparticles were nanometric in size and the particle size was found to less than 20 nm in both plant extracts and silver nanoparticles. The SEM image revealed the morphology of the particles. The plant extracts and synthesized silver nanoparticles using aqueous solution of plant extract shows good antibacterial efficacy against pathogens. Thus the antimicrobial activities of silver nanoparticles were established against Klebsiella, Pseudomonas aeruginosa, Staph aureus and Bacillus Cereus. In the future, it would be significant to know the precise mechanism of biosynthesis and to technologically engineer the nanoparticles with the aim of attaining better control shape, over size and whole monodispersivity.

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