

**A GREEN APPROACH FOR THE
SYNTHESIS OF STABLE SILVER
NANOPARTICLES USING *KALANCHOE
BLOSSFELDIANA* AND THEIR
CHARACTERIZATION**

**A Thesis Submitted
In Partial Fulfillment of the Requirements for the
Degree of**

MASTERS OF SCIENCE

**In
Physics**

by

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**Under the Supervision of
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A green approach for the synthesis of stable silver nanoparticles using *Kalanchoe blossfeldiana* and their characterization

Devangana Ghosh

ABSTRACT

A green approach for the synthesis of nanoparticles has attracted significant attention owing to its advantages including single-step synthesis process, rapidity, environmental friendliness, and cost-efficiency. The genus *Kalanchoe* has a rich application in medicine, attributed to its notable therapeutic properties. Various species within this genus share both chemical and physical traits. This study focuses on a particular species of *Kalanchoe-Kalanchoe blossfeldiana*. Stable silver nanoparticles (AgNPs) were synthesized through a green approach using the *Kalanchoe blossfeldiana* extract as a reducing agent. The synthesized AgNPs underwent various characterization to study their physical, chemical as well as biological properties. They were confirmed through multiple techniques including UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and transmission electron microscopy (TEM). The XRD peaks obtained confirm the crystalline nature of the biosynthesized silver nanoparticles. The crystallite size of the synthesised silver nanoparticle is 18.02nm. The surface charge potential of the biosynthesized AgNPs is -12.6 mV showing good stability with average particle size is around 29.6 nm. Thus, stable AgNPs of different sizes range from 10 to 35 nm were formed. The absorption spectra were recorded with wavelengths ranging from 280 to 700 nm. The AgNPs showed their maximum absorption peak at around 451 nm. The average size of the synthesized AgNPs is 21 nm.

Keywords: Green synthesis, silver nanoparticles, bioreductors, *Kalanchoe blossfeldiana*, SPR.

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Chapter 1

LITERATURE REVIEW

1.1 Introduction

The field of science is experiencing a miniaturization revolution. At the forefront lies nanotechnology, a blooming field where manipulations are done on the matter at atomic and molecular levels. The nanoscale materials exhibit significantly different characteristics and behaviors than microscale materials. At the intersection of physics, chemistry, biology, and engineering lies nanotechnology [1], a realm where scientists wield atoms to create entirely new materials with extraordinary properties.

The term "nano" derives from the Greek language, meaning "dwarf" or "something incredibly small"[2]. A nanometer (nm) is equivalent to one billionth, or 10^{-9} , of a meter[3]. As conventionally defined by the National Nanotechnology Initiative, United States, nanotechnology comprises a scale range of 1 to 100 nm.

Nanoscience is the study of extremely tiny entities such as structures and molecules, with dimensions ranging from one to hundred nanometers[2]. Nanotechnology is the area that applies this knowledge to create new entities. The combination of both fields has led to huge discoveries that is not only beneficial to mankind but also open a path to sustainability. The relentless exploitation of the nature has led to the destruction of the ecosystem, so it is extremely important to shift our concern towards preservation and conservation of the nature for its sustainability.

Nanoionics, focusing on diffusion, reactions at the nanoscale, and materials with fast ion transport, is a significant aspect. Understanding the mechanical properties of nanosystems is a key focus of nanomechanics, additionally, the interaction between nanomaterials and biomaterials can pose potential risks due to their catalytic activity. At the scale of the nano range, materials can display unique properties that allow for

special applications. As an example, certain substances that are typically opaque, such as copper, have the ability to transform into transparent material. Similarly, stable substances like aluminium can undergo a change and become combustible. Additionally, even insoluble materials like gold have the potential to become soluble under certain conditions. Even at normal scales, chemically inert materials like gold can exhibit powerful catalytic properties when reduced to the scale of nano range. There is a great fascination with the study of quantum as well as the surface phenomena that matter exists at the nanoscale, which is a major driving force behind the appeal of nanotechnology.

Above all, nanotechnology is a viewpoint, a way of seeing the world based on atomic precision. As such, it marks the pinnacle of man's insatiable need to comprehend the universe and apply that learning to practical ends. It is also known as "atomically precise technology" and encompasses the goal of designing "our earthly estate" atom-by-atom, regulating architecture, composition, and hence physical attributes with atomic precision.

1.2 History of nanotechnology

The application of nanotechnology emerged in the medieval period without a thorough understanding of the matter[4]. The Lycurgus cup, used during the fourth century AD, is a dichroic glass, considered one of the oldest synthetic nanocomposites, shows the aesthetic beauty and attractiveness of gold or silver nanoparticles[4]. This remarkable work with glass made by the Romans is one of the masterpieces of advanced nanotechnology in antiquity. The Lycurgus cup, part of the British Museum's collection, had shown a surprisingly unique phenomena, it undergoes chromatic variations in response to different light sources, seems green when light falls on the outside, but red when lighted from the inside, and King Lycurgus appears purple[5]. The scientific root cause for this effect finally came to light only in the year 1990, when atomic force microscope used for an detailed study of the cup by the scientists[5]. It was discovered that a phenomenon known as dichroism (two colours) is caused by the presence of silver, gold and copper nanoparticles ranging upto a size

of 100 nm is present in a glass matrix[5]. These metal nanoparticles present in the glass matrix absorb different wavelengths of light that cause a colour change such as ruby red colour is due to gold particles, purple is due to large particles of gold and green is due to silver particles[5]. The investigation on this interesting interaction between light and matter began around mid-1850 by Michael Faraday who first recognized the ruby red colour is due to the reduction of gold chloride, which scatters light but Gustav Mie in 1908 finally gave the theoretical explanation of the absorption and scattering of spherical colloidal particles[6].

Ceramic glazes were used in 9th to 17th century by the Islamic community and later in Europe which had a glowing, glittering, and luster property containing silver, copper, or other nanoparticles, and also showed optical behaviour[7]. A variety of layers of metallic thin film were decorated by applying on the surface of these ceramic glazes which causes lustre[8].

The European cathedrals during the period of 6th to 15th centuries had vibrant stained-glass windows having rich colours of gold chloride nanoparticles and other metal oxides which not only aesthetically beautiful but also acted as a photocatalytic air purifier[9].

During the thirteenth to eighteenth centuries, Damascus saber blades were used to fight against Muslims having high strength, resilience, and sharp cutting edge and gave a visible extraordinary beautiful pattern in the blade of ultrahigh carbon steel, containing rare earth elements, carbon nanotubes, and cementite nanowires[10].

The discoveries and developments in nanotechnology in the modern era gave proper explanations and theories to the premodern nanotechnologies. The Nobel laureate Michael Faraday in 1857 discovered the colloidal ruby red gold showing the behaviour of nanoparticles of gold, that is changes its colour when exposed to light [11]. In 1936 Erwin Muller invented a field emission microscope which made it possible to study the atomic-level resolution of images of materials. Finally, in 1959, Richard Feynman gave his first lecture on atomic-scale engineering, "There's Plenty of Room at the Bottom" during the annual meeting of the American Physical Society at Caltech[2], leading to the emergence of nanotechnology, establishing him as a pioneer in this field, as the father of modern nanotechnology[2]. A Japanese scientist named Norio Taniguchi for the first time used and defined the term "nanotechnology"

in the year of 1974 which is almost 15 years later of that talk. After this, there were several discoveries and advancements made in this field and still, many researches are going on. In 2023, Nobel prize in Chemistry was awarded for the work in the synthesis of quantum dots.

1.3 Classification of Nanomaterials

On the basis of dimensions, nanomaterials are classified into various categories. Nanomaterials to be in nano-dimension must possess at least a dimension that falls within the range of 1 to 100 hundred nanometers. By altering the dimensions, a variety of nanomaterials can be formed.

When nanomaterials having all three dimensions lie in the nano range, such as fullerenes, quantum dots, nanoparticles, polymer dots, nanospheres, and nanoclusters, they are called zero-dimensional (0D) nanomaterials. When any two dimensions are in the nano range, such as nanorods, nanowires, and nanotubes, they are called one-dimensional (1D) nanomaterials. When there is only one dimension is in the nano range, such as nanosheets, graphene derivatives, and quantum well, are called two-dimensional (2D) nanomaterials. Any bulk nanomaterial which has all the dimensions more than 1 to 100 nanometer such as nanowire bundles, nanotube bundles, and multi-nanolayers, are called three dimensional (3D) nanomaterials[12]. Fig. 1 represents an illustration of the categorization of nanomaterials on the basis of their dimensions.

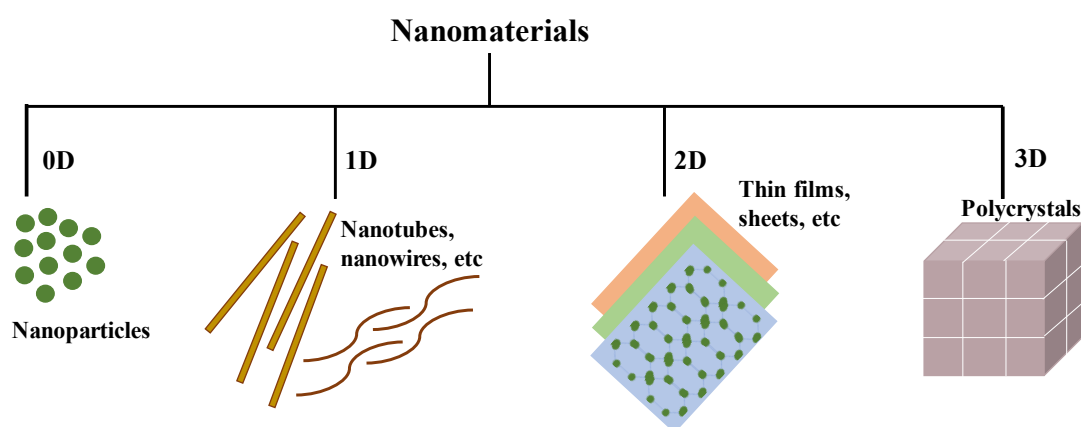


Fig 1: Classification of nanomaterials on the basis of dimensions

1.4 Nanoparticles

Nanoparticles are minute particles with its sizes ranging from 1 to 100 nm possess two unique properties that are, the ratio of surface area and volume, which increases with the decrease in the particle size, and quantum confinement which is the spatial confinement of excited electrons and associated holes which is also referred as exciton, in one or more dimensions within a material. When it comes to nanoparticles of a material, their small size gives them unique physical, chemical, and biological properties that set them apart from the larger particles of the same material[11]. These distinct properties possess attractive qualities suitable for developing nano biosensors, probes, therapeutic agents, energy storage, vaccines, drug deliveries, and eliminating toxins.

Exposing plants to nanoparticles (NPs) can significantly influence their physiological processes, leading to heightened metabolic rates and accelerated germination, growth, and development.[13] Additionally, Nanoparticles introduce novel mechanisms for plant protection, initiating the activation of antioxidant enzymes and promoting enhanced plant regeneration[13].

1.5 Types of Nanoparticles

Nanoparticles are primarily grouped into two categories namely inorganic nanoparticles and organic nanoparticles. There are many other types of nanoparticles that are represented in fig. 2.

1.5.1 Inorganic nanoparticles

The inorganic nanoparticles such as the metal, magnetic, ceramic and semiconductor nanoparticles.

Silver, gold, copper, aluminum, lead, and many other metals are examples of metal nanoparticles, having remarkable electrical and optical properties which could

be ascribed to surface plasmon resonance[14]. The pure metal nanoparticles are obtained from the metal precursors.

Magnetic nanoparticles include cobalt, iron, and nickel. These nanoparticles possess some properties like uniformity in size, shape, high surface-to-volume ratio, biocompatibility, superparamagnetism, and magnetic moment causing hugely increase in biomedical applications such as drug delivery[15], gene therapy[15], targeted cancer treatment[15], and biotechnological applications[16]. These nanoparticles are primarily utilized to facilitate the separation, purification and concentration of various biomolecules. Magnetite (Fe_3O_4) and Maghemite (Fe_2O_3) are the most biocompatible magnetic nanoparticles[17].

Ceramic nanoparticles are mainly consisting of oxides, nitrides, or carbides. These nanoparticles are widely used for coatings because of their heat resistance and chemical inertness[18]. These nanoparticles possess high mechanical strength, temperature resistant, high stability, high load capacity, and ease of incorporation into hydrophobic and hydrophilic systems[18]. At present, the development of extraordinary ceramic materials for biomedical applications is proceeding rapidly[19]. But it comes with some limitations such as high density, low biodegradability, and high toxicity. Hydroxyapatite (HA), Zirconia (ZrO_2), Silica (SiO_2), Titanium oxide (TiO_2), and Alumina (Al_2O_3) are developed from the new methods for synthesis to enhance their physical and chemical characteristics and lessen their cytotoxicity in biological systems[19]. Some other additional applications of ceramic nanoparticles includes drug delivery, tissue engineering, and biosensors.

Semiconductor nanoparticles include zinc oxide and zinc sulfide. These nanoparticles show both metallic and non-metallic properties, with broadband gaps. These nanoparticles are commonly used in photocatalytic and electronic devices[12]. It has been observed that metal oxide nanoparticles show better reactivity and efficiency than that of metal nanoparticles[20].

1.5.2 Organic nanoparticles

Organic nanoparticles are carbon nanotubes, quantum dots, and carbon-based

nanoparticles. Carbon-based nanoparticles include fullerenes, graphenes, and carbon nanotubes[14].

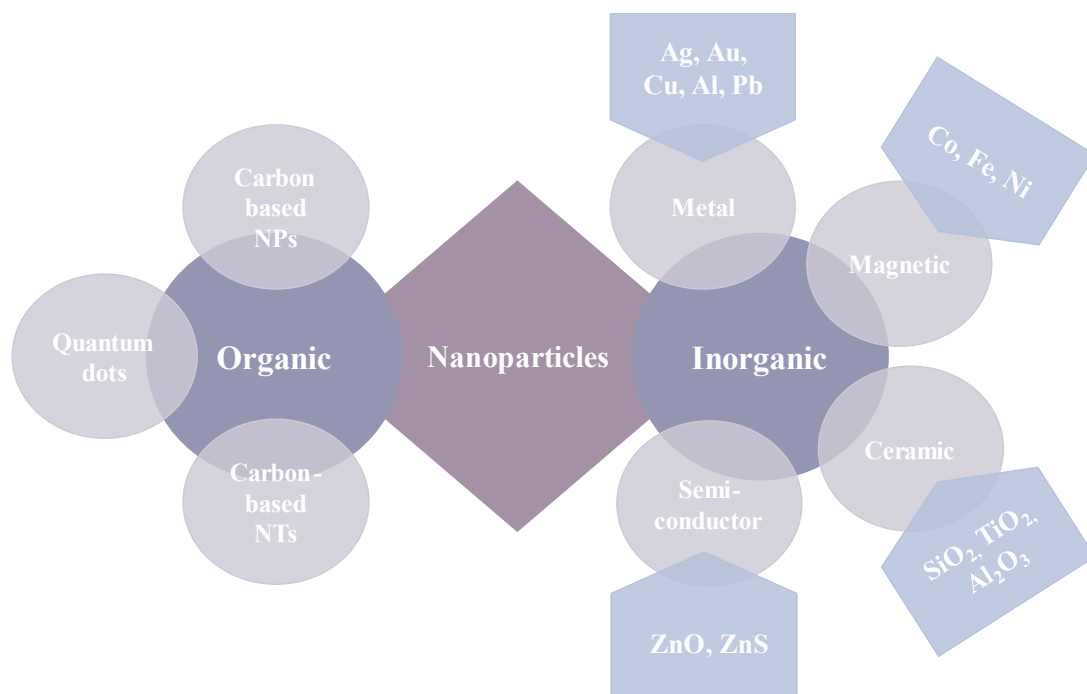


Fig. 2: Nanoparticles and their types

1.6 Silver nanoparticles

Silver, a gleaming metal coveted for centuries for its beauty, malleability, and ductility boasts a surprising arsenal of properties that extends far beyond aesthetics. Silver, known for its thermal and electrical conductivity, has a wide range of uses, particularly in medicine owing to its intrinsic antimicrobial, antibacterial, antifungal, and antioxidant characteristics[7,8]. The relentless march of nanotechnology has propelled silver nanoparticles, a noble metal nanoparticle, to the forefront of scientific exploration owing to their exceptional properties in diverse areas and disciplines like medicine, catalytic activity, and electronics [22].

Among the various metallic nanoparticles developed to date, silver and its nanoparticles have drawn significant heed because of their versatile and budding applications in various sectors including optoelectronics, environmental remediation, electrochemistry, textile industries and biomedicines [23]. However, the conventional

chemical synthesis methods for silver nanoparticles often leave a trail of environmental devastation due to their reliance on toxic chemicals and hazardous byproducts, and also a time-consuming process. This escalating environmental concern has fueled the quest for sustainable alternatives.

1.7 Approaches for the synthesis of nanoparticles

Following the impactful lecture of Feynman, scientists started looking for different possible approaches for developing and synthesizing nanoparticles. There are two distinct approaches followed for the synthesis of nanoparticles: the top-down approach and the bottom-up approach. Fig. 3 depicts the different synthesis processes of nanoparticles.

1.7.1 Top-down approach

The top-down approach is basically a destructive approach in which the breakdown of bulk matter to obtain particles of nano range. This approach involves various physical and chemical techniques for the synthesis of nanoparticles. These physical and chemical methods include inert gas condensation, electron beam lithography, ball milling, laser ablation, vapour phase synthesis, pyrolysis, chemical etching, sputtering, and many more[24]. This method is fast and scalable but achieving particular properties and shapes can be challenging.

1.7.2 Bottom-up approach

The bottom-up approach is a method in which the process starts with atoms or molecules to end up with the formation of nanoparticles[25]. This method includes chemical methods, like coprecipitation, sol-gel process, microemulsion, and many more, and biological methods using plant extracts, microorganisms, enzymes, and

many other biological products and wastes as bioreductors for the synthesis of nanoparticles[11].

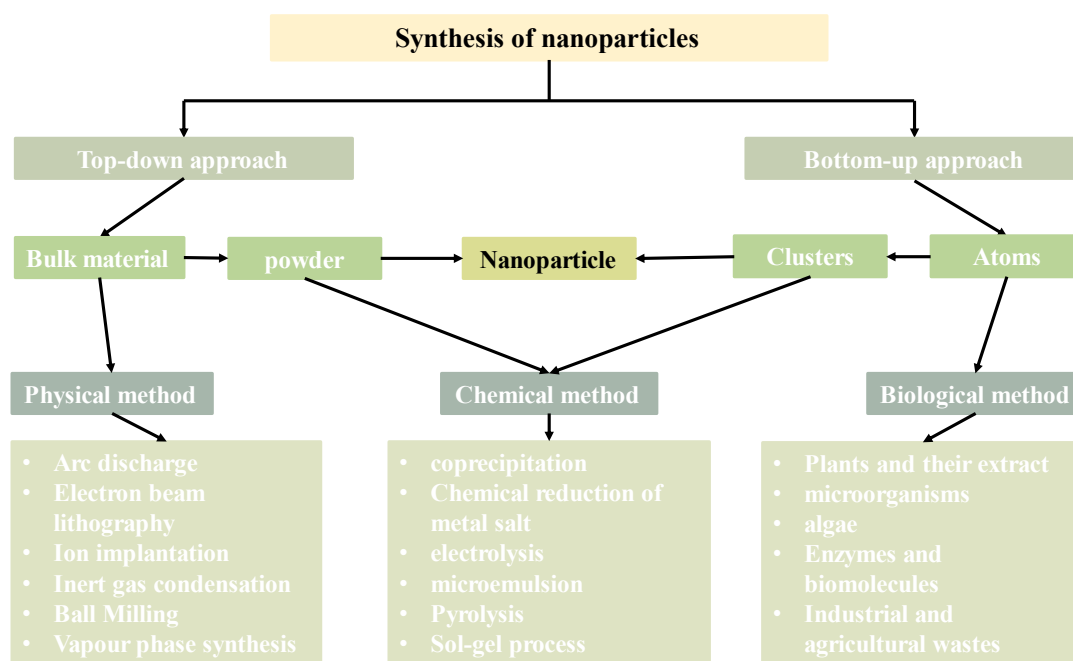


Fig. 3: Various synthesis routes for nanoparticles

1.8 Biological method

The nanoparticle synthesis following a green approach uses bioresources like flower or leaf extracts, microbes, algae, biomolecules, enzymes, industrial wastes, and agricultural wastes, eliminating the requirement for harmful chemicals and waste products[26]. The extract obtained from leaves, flowers, fruits, roots, stems or any other part of a plant behaves like a reducing agent and also helps to stabilize the nanoparticle, acting as a stabilizing agent [27], leading to the formation of stable nanoparticles with controlled size and form. Fig. 1 shows the schematic illustration of green synthesis.

This eco-friendly approach not only protects the environment but also opens doors for the development of biocompatible nanomaterials with potentially unique properties. It is a low-cost, non-toxic, environmentally benign, scalable, and one-step

process[28]. By embracing green synthesis, we unlock the true potential of nanotechnology while ensuring a sustainable future. The green approach also referred to as biological synthesis methodology, which is a bottom-up approach [29] and this methodology is illustrated in Fig. 4.

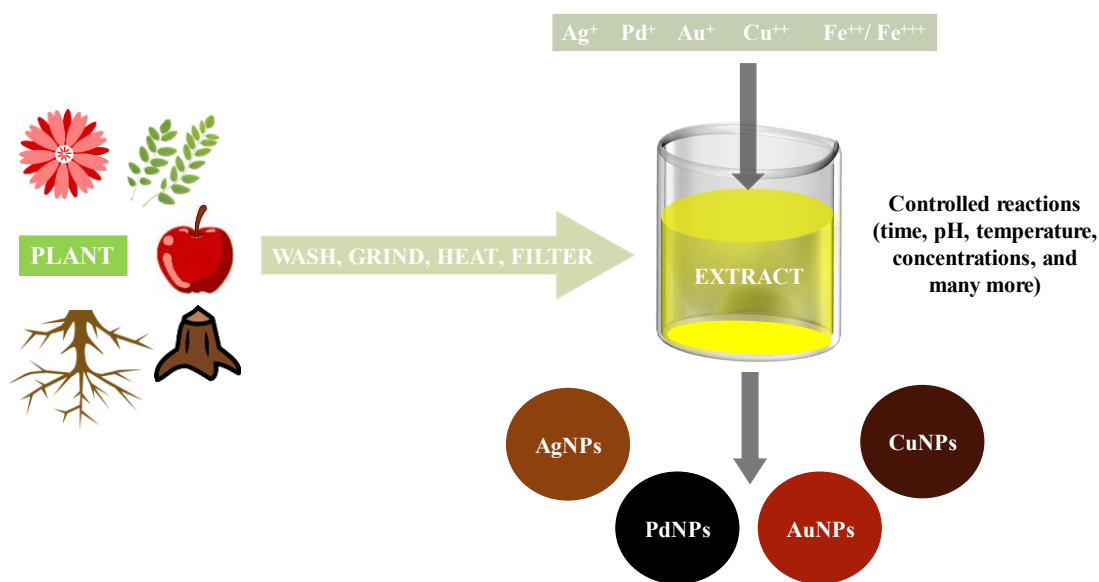


Fig. 4: Biological method for synthesis

1.9 Biological method over physical and chemical methods

Unlike conventional methods, nanotechnology has the potential to craft materials with new properties. This opens doors to a plethora of applications, from drug delivery in medicine to solar cells in energy production. The blooming field of nanotechnology has revolutionized various sectors, but traditional synthesis methods for nanoparticles cause harm to the environment. These traditional synthesis methods include physical and chemical methods that typically rely on harmful chemicals and generate hazardous byproducts, raising concerns about their sustainability. These synthesis methods are top-down approaches. These typical nanoparticle synthesis methods have some drawbacks such as limited solubility, suboptimal efficacy in therapeutic applications, and major environmental risks. The chemical methodologies for synthesis result in undesired byproducts and/or unreacted chemicals remaining in the synthesized

colloidal media, making them not at all suitable for medical use[30].

1.10 Surface plasmon resonance

When the incident photons come into contact with the conduction electrons of metal nanoparticles, it interacts with the conduction electrons of metal nanoparticles, and a phenomenon known as surface plasmon resonance occurs. Due to this, plasmons oscillate at a particular range of wavelengths which causes the metal nanoparticles to absorb and scatter light[31]. Because of their surface plasmon resonance properties, novel metal nanoparticles show bright-colored solutions [32]. This surface plasmon resonance property has been extensively used in numerous domains including spectroscopy, imaging, and sensing[32].

Chapter 2

A GREEN APPROACH

2.1 Plant extract as bioreductors

Plant extracts, brimming with bioactive compounds, present a promising avenue for sustainable synthesis using a green method of silver nanoparticles [33]. These reductants offer a sustainable and environmental friendly approach, abstaining from the use of harsh chemicals and minimizing waste generation[34]. Moreover, plant extracts behave as stabilizing agents affecting and altering the stability, size, and form of the biosynthesized silver nanoparticles[35].

The green approach involves using plant extract to reduce the Ag^+ to Ag^0 and this happens due to the existence of different phytochemicals in the plant such as amino acids, vitamins, proteins, terpenoids, enzymes, flavonoids, polyphenols, quinones, tannins, saponins, steroids, alkaloids, ketones, carboxylic acid and carbohydrates[36][37][38]. Though the exact reason for this reduction is still not properly understood and studies and research are still going on, but it was conjectured that Nicotinamide adenine dinucleotide hydrogen (NADH) coenzyme is responsible

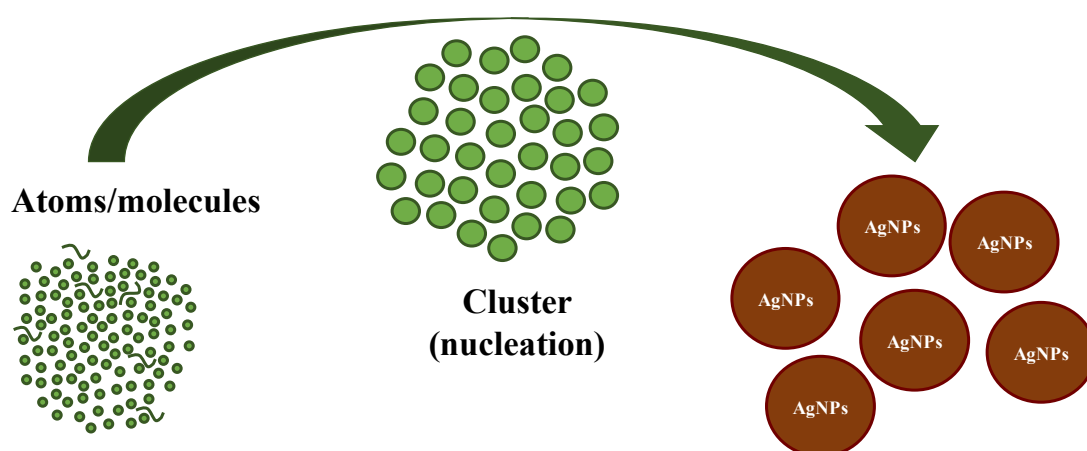


Fig. 5: Bottom-up approach for synthesis of silver nanoparticles

which acts as an electron shuttle that reduces the metals[39]. Fig. 5 shows the bottom-up green approach for the synthesis of silver nanoparticles.

2.2 The genus *Kalanchoe*

The genus *Kalanchoe* belongs to the genus *Crassulaceae*. The species of this genus are popularly known as Christmas plants. The *Kalanchoe* species are succulents majorly found in tropical areas. These species have various colourful flowering plants making them widely used for decorating and ornamenting. *Kalanchoe* is easy to grow and is also considered the easiest and fastest plant to reproduce in the world, making it a widely accessible blooming and flowering plant [40]. The phytochemicals that are majorly responsible for reducing silver for the synthesis of silver nanoparticles are flavonoids, alkaloids, tannins, steroids, iridoids, proteins, reducing sugar, polyphenols, glycosides, saponins, and terpenes [41].

2.3 *Kalanchoe blossfeldiana*

Kalanchoe blossfeldiana, also known as florist *Kalanchoe* has vibrant flowers of different colours from reds to oranges to whites. This plant is an ornamental and also medicinal plant. The majorly found phytochemicals in *Kalanchoe blossfeldiana* are flavanol glycosides which are derived from quercetin, kaempferol, or gossypetin, gallic acid, and proanthocyanidins [42]. Fig. 6 shows the plant used in this study: *Kalanchoe blossfeldiana*.

There have been multiple studies conducted extensively on the synthesis of silver nanoparticles (AgNPs) utilizing a variety of ecologically sustainable green sources[43]. Synthesis of AgNPs using extract of *Rhodiola rosea*[44], *Nephrolepis radicans*[45], *Zingiber officinale*[1], *Punica granatum*[46], *Acalypha indica*[46], *Ficus benghalensis*[46], *Galenia africana*[46], *Terminalia mantaly*[46], *Kalanchoe pinnata*[47], *Euphorbia serpens*[48], *Aeonium haworthii*[49], *Dudleya brittonii*[50], *Sedeveria pink ruby*[30], *Phyllanthus urinaria*[51], *Pouzolzia*

zeylanica[51], *Euphorbia Pseudocactus Berger*[52], *Ochradenus arabicus*[13], *Maerua Oblongifolia*[13], *Scoparia dulcis*[51], *Stachytarpha indica*[53], *Munronia pinnata*[53], *Rhipsalis baccifera*[53], *Syngonium podophyllum*[54], *Ferocactus echidne*[55], *Catharanthus roseus Linn. G. Don*[56], *Trianthema decandra*[57], *Euphorbia milii*[58], *Elettaria Cardamomom*[59], *Kalanchoe fedtschenkoi*[1], *Annona senegalensis*[36], *Argyreia nervosa*[60], *Cynara scolymus*[61], *Plantago lanceolata*[62], *Alternanthera Sessilis*[63], *Kalanchoe gastonis-bonnierii*[64], *Kalanchoe Daigremontiana*[65], *Kalanchoe petitiiana A. Rich*[66], and many more are reported.



Fig. 6: *Kalanchoe blossfeldiana*

This study focuses on the biosynthesis of stable silver nanoparticles and this biosynthesis follows a green methodology by the use of extract made from leaves of *Kalanchoe blossfeldiana* as there are many studies on different species of *Kalanchoe* but there is till date no study on this particular species. The highly stable synthesized silver nanoparticles are further being investigated which enable its use in various medicinal fields. The growing industrialization often leads to harm to the environment which increases the exposure of humans to various heavy toxic metals leading to various health issues especially cancer and silver nanoparticles can be used for treatment. Green technology-derived nanoparticles exhibit diverse properties, exceptional stability, and optimal sizes due to their one-step production process. Unlike traditional synthesis methods involving toxic chemicals on the surface, the use of green techniques eliminates this concern.

Chapter 3

EXPERIMENTAL

3.1 Materials

AgNO₃ was purchased from Sigma-Aldrich. The plant used to carry out this experiment, *Kalanchoe blossfeldiana* was provided by the nursery of Delhi Technological University, New Delhi. All the experiments performed in this present study utilized deionized water with a specific resistance of 18.2 MΩ cm. Whatman filter paper no. 1 was used to filter the leaf extract.

3.2 Preparation of leaf extract

The pristine leaves of *Kalanchoe blossfeldiana* were carefully picked and meticulously rinsed with normal water in order to eliminate soil, dirt, and contaminants. These leaves were then rinsed with deionized (DI) water and allowed to

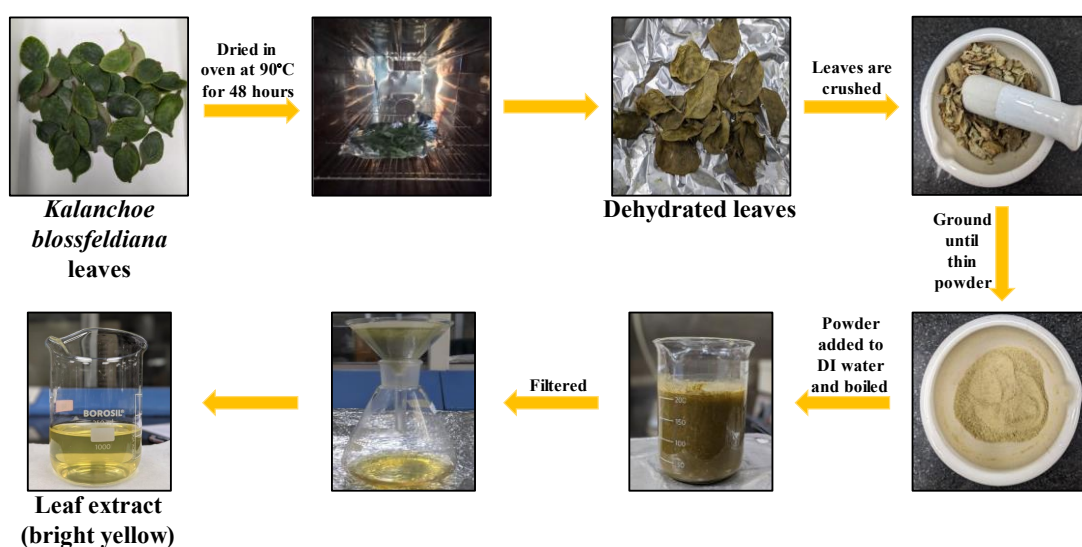


Fig. 7: Preparation of *Kalanchoe blossfeldiana* leaf extract

dry in the air. The leaves were then dried in the oven at a temperature of 90 °C for approximately 48 h. The dehydrated leaves are then pulverized using a porcelain mortar and pestle. A fine powder is obtained. In a beaker, 2.0 g of this powder is added to 25 mL of deionized (DI) water and stirred at a temperature of 90 °C and 450 rpm for about 30 min. After it cools down, the liquid is poured on filter paper using Whatman filter paper no. 1. The filtering process a time taking and took approximately around 8 h. Finally, a bright yellow extract is obtained. The leaf extract is stored at a temperature of 4°C in the refrigerator for later use [67]. Fig. 7 depicts a schematic illustration of the leaf extract preparation. This extract is further used for developing silver nanoparticles [68].

3.3 Biosynthesis of silver nanoparticles

The biosynthesis of AgNPs begin with the preparation of a 1 mM solution of silver nitrate (AgNO_3). To start with, for one millimolar solution to prepare, one mole of silver nitrate is taken in a butter paper, that is 17mg of AgNO_3 is weighed using a weighing machine. Then in a beaker, this 17 mg of AgNO_3 is added to 100 mL of deionised water (DI) to prepare 1 mM of AgNO_3 solution. The solution is shaken to mix it thoroughly. Now, from that, a small amount (10 mL) of 1 mM AgNO_3 solution is taken out in a beaker and is stirred at a temperature of 50 °C with 300 rpm for a duration of 15 min. After it cools down and reaches around the room temperature, 2 mL of prepared *Kalanchoe blossfeldiana* leaf extract is added dropwise using a

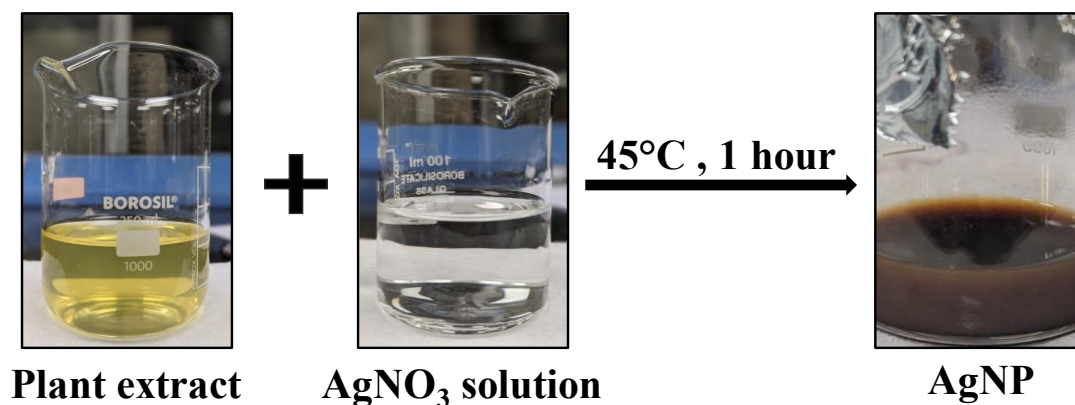


Fig. 8: Formation of silver nanoparticles

dropper to that 1mM AgNO₃ solution. This process is also maintained at a temperature of 45°C with 400 rpm in a stirrer. As the extract is added to the AgNO₃ solution, an alternation in the colour of the solution is observed. Fig. 8 depicts the formation of AgNO₃ solution by adding *Kalanchoe blossfeldiana* leaf extract. The colour of the solution changes over time from translucent white solution to yellow to yellowish orange to finally dark reddish-brown colour [69] and is depicted in Fig. 9. The dark reddish-brown colour confirms the formation of AgNPs.

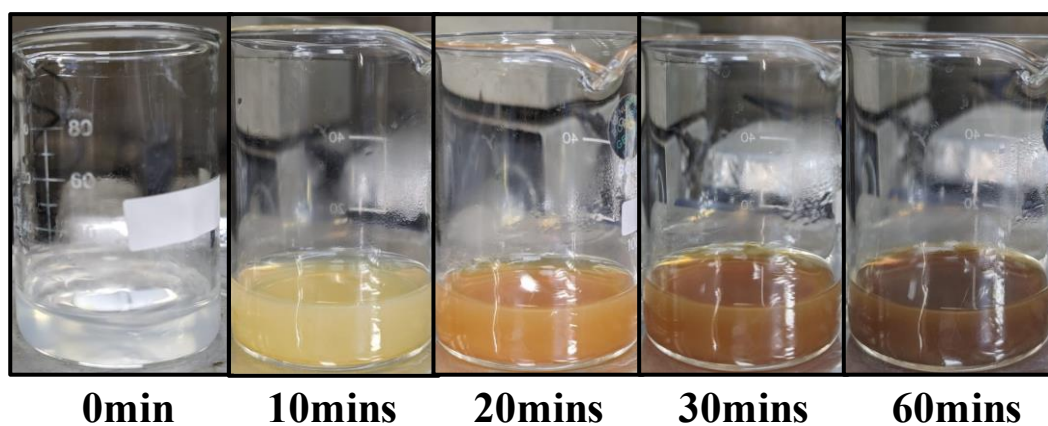


Fig 9: Gradual colour change of silver nanoparticles with time

Chapter 4

INSTRUMENTATION

Lambda 365+ UV/VIS spectrometer from Perkin Elmer was used for obtaining absorption spectra, Bruker's D-8 Advanced was used for obtaining X-ray diffraction pattern (XRD) pattern, FEI Tecnai F30 FEG was used for obtaining TEM images and Fourier transform infrared (FTIR) spectra were measured with a FT-IR Spectrometer Spectrum two from PerkinElmer. Malvern Zetasizer was used to assess the zeta potential of the biosynthesized silver nanoparticles.

Chapter 5

CHARACTERIZATION TECHNIQUES

5.1 X-ray diffraction pattern

The X-ray diffraction study of biosynthesized nanoparticles is relatively a new use of this technique to analyze the properties of the biosynthesized nanoparticles[70]. X-ray diffraction patterns were obtained to analyze the crystallinity and nanostructures of the biosynthesized stable silver nanoparticles by preparing thin films by the method of drop coating technique[71,72] In general widening of peaks in the XRD patterns indicates the decreasing size of the particle and reflects the influence of experimental circumstances on the nucleation and growth of the crystal [73].



Figure 10: Bruker's D-8 Advanced

The constituents of the particles can be verified by comparing the orientation and intensity of the peaks to reference patterns from the International Centre for Diffraction data (ICDD) formerly known as the Joint Committee on Powder Diffraction Standards (JCPDS) database[74].

Debye Scherrer's equation, $D=K\lambda/(\beta\cos \theta)$ helps to determine the crystallite size, where K is the Scherrer's constant whose value varies from 0.9 to 1, λ is the wavelength of the X-ray, β is the full-width half maxima (FWHM) and θ is the Bragg's diffraction angle and is calculated in radians. Fig. 10 is the instrument used for XRD.

5.2 UV-vis absorption spectra

UV-visible spectroscopy is used extensively for characterizing the nanoparticles[75]. Also, this is the very first characterization done to confirm the formation of nanoparticles. The spectra obtained from the UV-vis spectroscopy depict the phenomenon of surface plasmon resonance at a particular range of wavelength which is different for different nanoparticles and this ultimately corresponds to the formation of nanoparticles[39]. The maximum peaks depict the maximum absorbance, where nanoparticles absorb maximum radiations due to electron transitions, at a particular wavelength which is different for different nanoparticles[39]. The instrument used in



Figure 11: Lambda 365+ UV/VIS spectrometer from Perkin Elmer

this study to absorption spectrum, Lambda 365+ UV/VIS spectrometer from Perkin Elmer is shown in Fig. 11.

5.3 Zeta Potential

The electrostatic repulsion or attraction between the particles is the zeta potential indicating the surface charge potential[61]. It is a physical characteristic of nanoparticles that determines the net surface charge of the nanoparticles [60], and is an essential and important metric for studying the stability of the nanoparticles in aqueous solutions [61]. The large values of negative or positive zeta potential indicate higher and stronger electrostatic repulsiveness between the nanoparticles with zero propensity for flocculation [32]. The stability of nanoparticles is assessed using zeta potential values ranging from +30 mV to -30 mV [76].



Figure 12: Malvern Zetasizer

Dynamic light scattering (DLS) is an ensemble measurement used for estimating the average hydrodynamic diameter of a particle[77]. DLS is intrinsically sensitive to the presence of larger particles within a sample because the intensity of the scattered light depends on the size of the particles[77][78]. This might explain the observed increase in the average particle size compared to the crystallite size[78]. Malvern Zetasizer was used for this study, shown in Fig. 12.

5.4 HRTEM analysis

Nanoparticles exhibit a range of sizes and forms and each of these sizes and forms shows different physical and chemical properties. Green synthesis generates a huge range of sizes and forms and is studied with the help of a size distribution curve[79]. High-resolution transmission electron microscopy (HRTEM) images give a high-resolution image of nanoparticles of different sizes and forms that helps to study the nanoparticles thoroughly[80]. It is worth noting that a significantly huge portion of TEM research is focused on plant-mediated green synthesis of nanoparticles[79].



Figure 13: FEI Tecnai F30 FEG

Selected area electron diffraction (SAED) is done using a transmission electron microscope is a crystallographic experimental method[81]. The SAED technique, also known as the electron diffraction method in TEM, assesses various lattice parameters from a variety of diffraction patterns to determine the crystalline structure of nanoparticles[82]. This approach, which uses a parallel beam of high-energy electrons to aim at a tiny sample, also determines crystal structure, and crystallinity extent[83]. FEI Tecnai F30 FEG is used for obtaining HRTEM images, shown in fig. 13.

5.5 FTIR analysis

FTIR analyses are done for identifying biomolecules that may effectively stabilize metal nanoparticles[84] and for determining the chemical composition of the metal nanoparticles present on its surface[85]. The FTIR spectrum shows the existence of active functional groups in the synthesized nanoparticles[59]. Every peak in the FTIR spectrum having different wavenumbers shows bond stretching, indicating the presence of various molecules. The instrument used for performing FTIR is FT-IR Spectrometer Spectrum two from PerkinElmer, is shown in Fig. 14.



Figure 14: FT-IR Spectrometer Spectrum two from PerkinElmer

Chapter 6

RESULTS AND DISCUSSION

6.1 X-ray diffraction pattern

The obtained XRD pattern of the biosynthesized silver nanoparticles was further investigated and plotted using origin software (OriginPro 2024)[71], which is shown in Figure 3. Four major distinct Bragg's diffraction peaks (2θ value) were found around 38.22° , 46.24° , 54.92° , 67.44° and 76.9° representing lattice planes (111), (200), (211), (220), and (311) respectively (JCPDS file no. 84-0713 and 04-0783)[86].

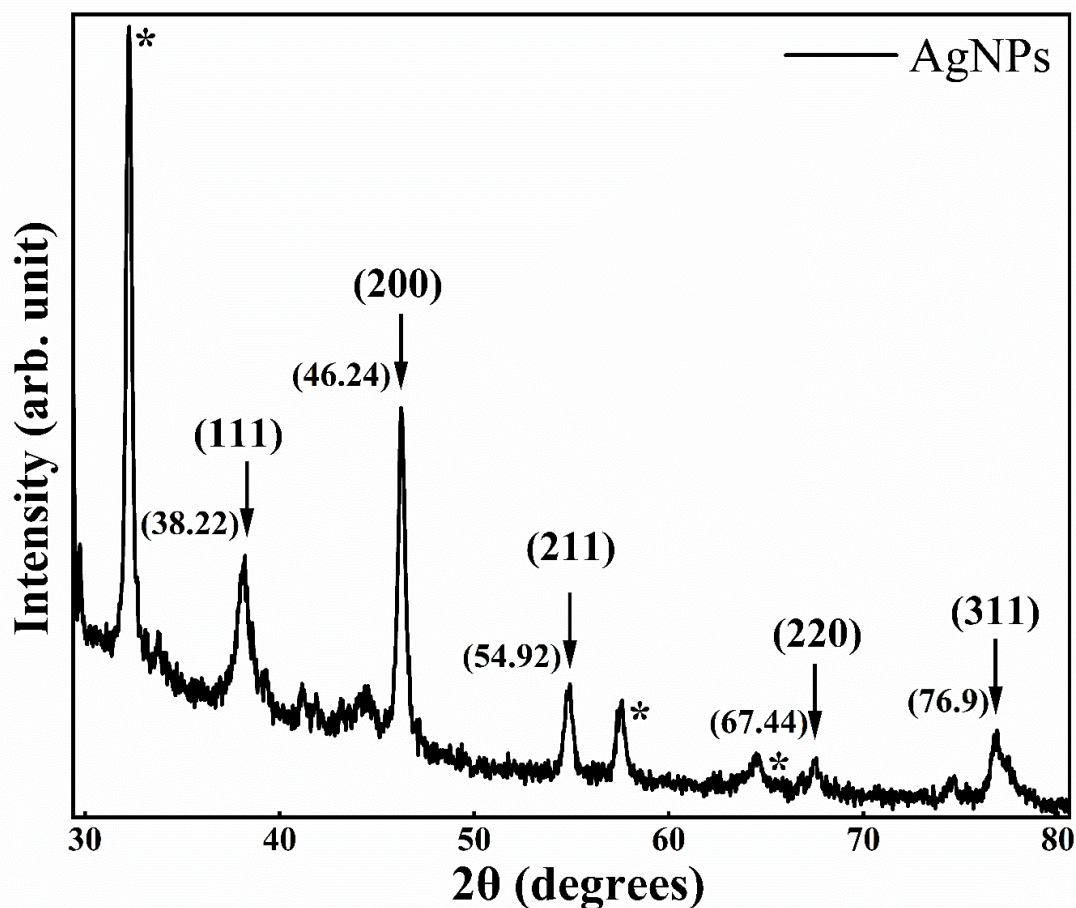


Fig. 15: XRD pattern of biosynthesized silver nanoparticles thin film

These diffraction peaks and planes depict and confirm having a face-centered cubic lattice structure [87]. The calculated average particle size using Scherrer's formula has come out to be 18.02nm. The unidentified peaks marked with asterisk marks near 32.24°, 57.6°, and 64.5° indicate the presence of the bio-organic phase on the surface of the nanoparticles[88]. Thorough research is still going on to study more about this crystalline phase that coexists in silver nanocrystals [89]. Fig. 15 shows the XRD analysis of the formed silver nanoparticle thin film.

6.2 UV-vis absorption spectra

The formed biosynthesized stable silver nanoparticle was validated by measuring the absorption spectra. The maximum absorption band lies in wavelengths

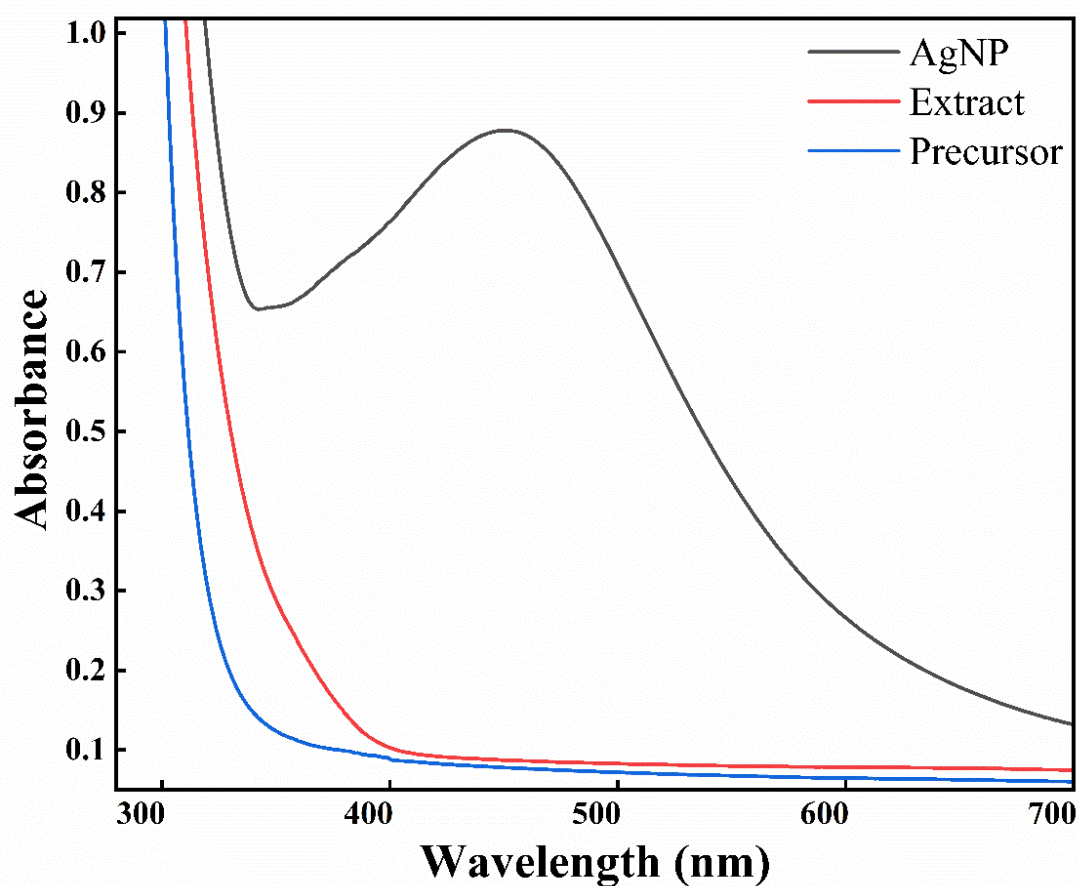


Fig. 16: Absorption spectra for biosynthesized silver nanoparticles along with precursor and leaf extract

ranging from 300 to 700 nm[90]. Due to distinctive optical characteristics referred to as surface plasmon resonance (SPR), the UV-visible spectrophotometer is the most practical and useful instrument for monitoring the conversion of silver ions to silver nanoparticles due to a reduction in the charge from +1 to 0[91] and this bioreduction ultimately leads to a colour shift in the solution[92], indicating the formation of stable biosynthesized silver nanoparticles, showing a characteristic single and significantly strong absorption band at a wavelength of 451 nm. As the extract is added to the precursor, the solution starts to change its colour over time from yellowish white to yellowish orange and eventually to dark reddish brown. Fig. 16 shows the absorption spectra of precursor, leaf extract, and AgNPs.

For studying the effect of extract concentration, various concentrations of leaf extract used while maintaining the constant concentration of silver nitrate constant.

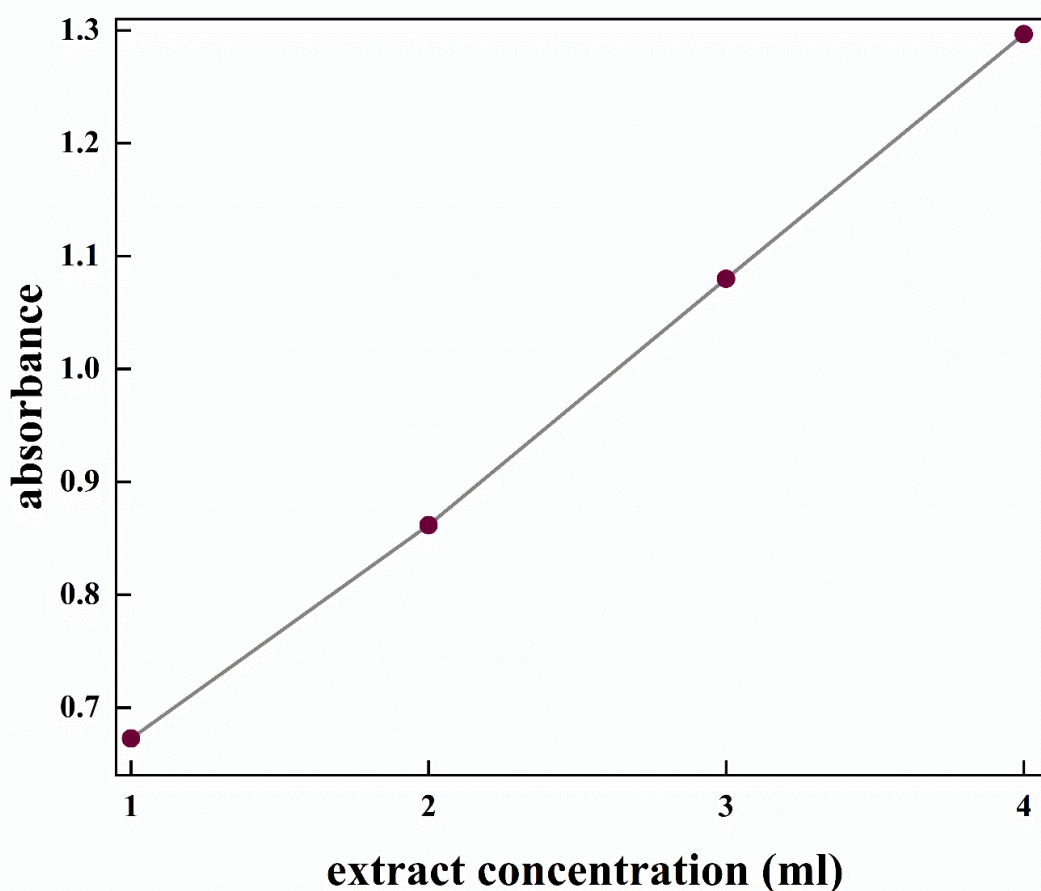


Figure 17: Absorption intensity at 451 nm with leaf extract concentration and absorbance

Four different distinct samples are prepared (1:10, 2:10, 3:10, and 4:10). Different concentrations of leaf extract (1ml, 2ml, 3ml, and 4ml) are added to silver nitrate (10ml). Though there is no noticeable and significant change in the colour of the solution observed, but the absorbance band differed for different samples. As the concentration of leaf extract solution increased, the absorbance increased[93]. The absorption spectra for the variation of leaf extract concentration with absorbance is shown in Fig. 17.

6.3 Zeta Potential

The average surface charge potential of the biosynthesized silver nanoparticle is measured to be -12.6 mV, as shown in fig. 18, indicating the good stability of the formed silver nanoparticles. The Fig. 19 shows the size distribution of particle with an average particle size of the synthesized silver nanoparticles around 29.6 nm.

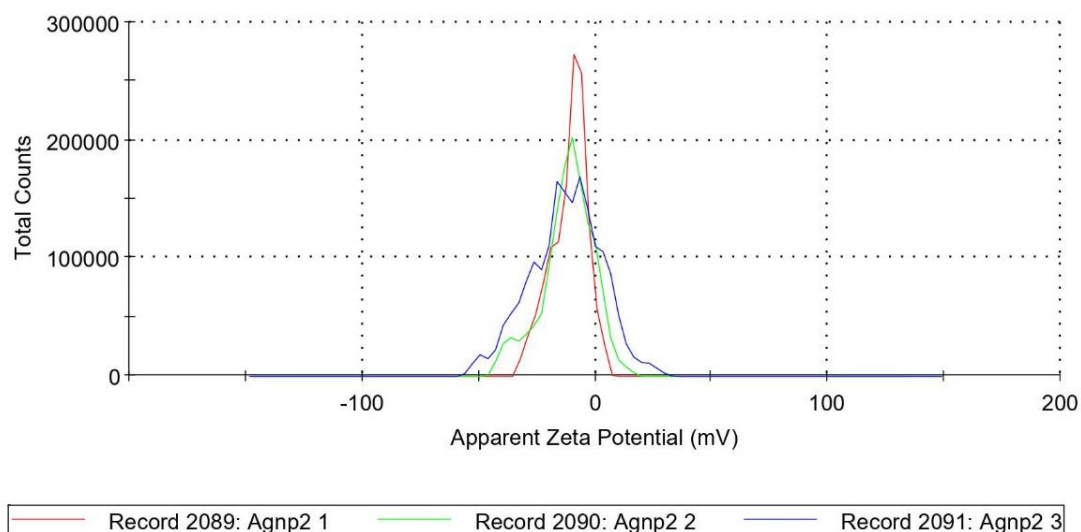


Figure 18: Zeta potential distribution

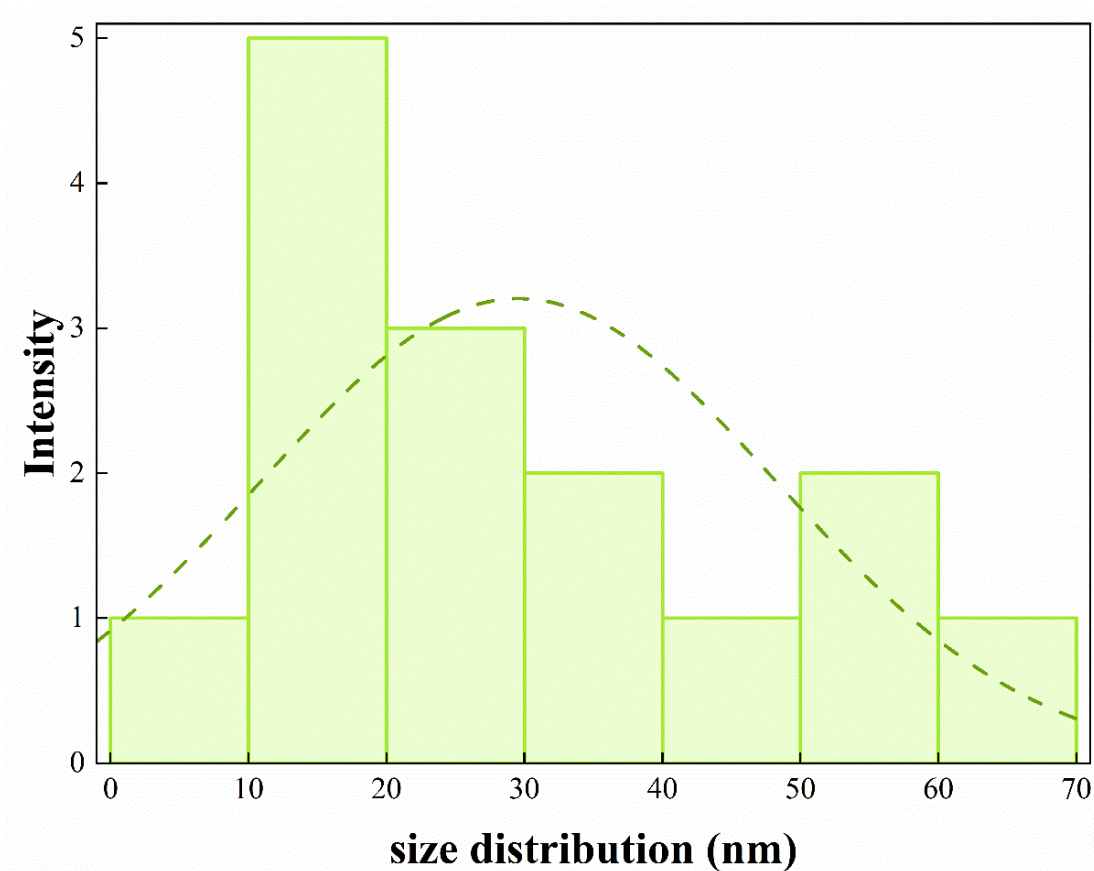


Figure 19: Size distribution of synthesized AgNPs obtained from DLS analysis

6.4 HRTEM analysis

HRTEM images obtained were taken to determine the size and form of the biosynthesized stable silver nanoparticles as it is one of the most sophisticated and advanced analytical high-resolution analyzing methods[94]. The spherical shape of the NPs was verified by using HRTEM images. A huge variety of different-sized nanoparticles were observed in the HRTEM images with diameters ranging from 10 to 35 nm. The HRTEM image is shown in Fig. 20. The average diameter of the biosynthesized silver nanoparticles is around 21nm. The selected area electron diffraction (SAED) pattern (inset, Fig. 20b) with circular rings corresponding to the

(111), (200), (211), (220) and (311) planes of face centred cubic (fcc) silver show the nanoparticles synthesized are highly crystalline[95].

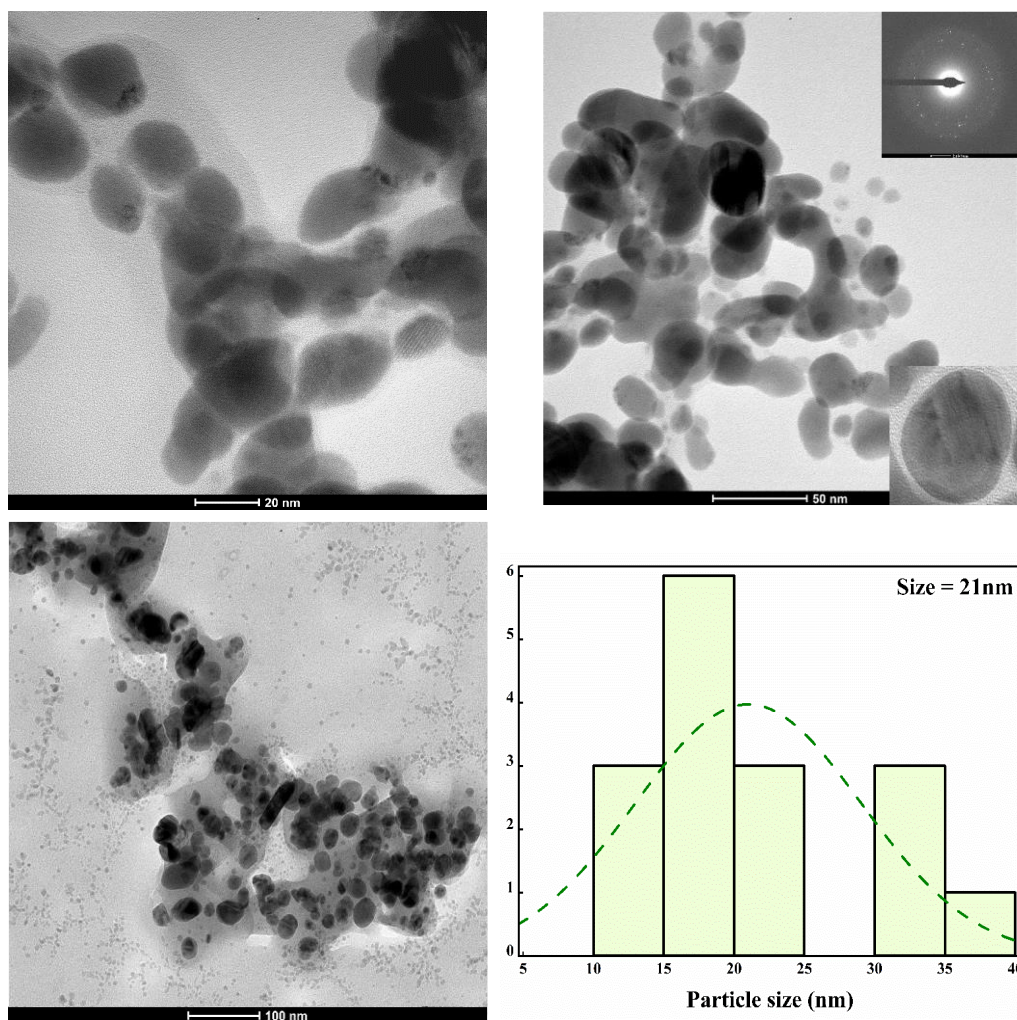


Fig. 20: HRTEM image of 20 nm (a), HRTEM image of 50 nm with SAED (b), and HRTEM image of 100 nm (c) along with mean diameter distance distribution (d) of biosynthesized silver nanoparticles

6.5 FTIR analysis

The characterization technique identifies the bonds between atoms of molecules present on the surface of the nanoparticle, with which the infrared light interacts. Fig. 21 shows the FTIR of synthesized stable silver nanoparticles with four

distinct peaks at 578, 1636, 2095, and 3312 cm^{-1} . These peaks depict different stretches of bond[96]. There are several biomolecules and phytochemicals that are present in the *Kalanchoe blossfeldiana* leaf extract which is responsible for reducing and stabilizing silver nitrate into silver nanoparticles [97]. The peak at 578 cm^{-1} corresponds to the alkyl halides stretch[98], 1636 cm^{-1} corresponds to C=O stretch[99], 2095 cm^{-1} corresponds to C=C stretch[26], and 3312 cm^{-1} corresponds to amine stretch[100].

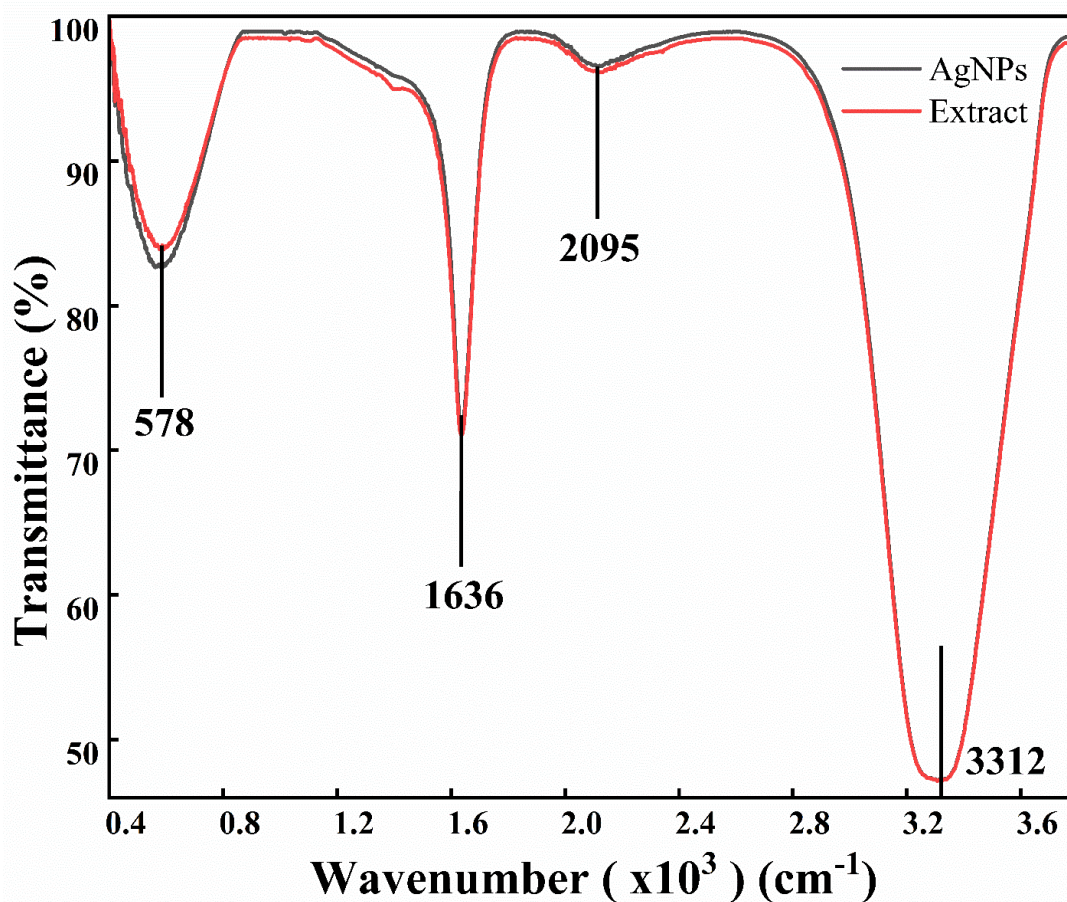


Fig. 21: FTIR spectra for biosynthesized silver nanoparticles and leaf extract

CONCLUSION

In this present study, stable biosynthesized AgNPs are successfully synthesized using a green technique. *Kalanchoe blossfeldiana* leaf extract served as both a reducing agent and a stabilizing agent. These nontoxic bioreductors have successfully reduced silver nitrate to form stable AgNPs. This green synthesis route is a sustainable, single-step, cost-efficient, and fast process. The biosynthesized stable AgNPs have a consistent spherical form with an average particle size of 21 nm. Stable AgNPs are synthesized with varying particle sizes that range from 10 to 35 nm. The biosynthesized AgNPs showed a strong surface plasmon resonance band at around 451 nm. As the extract concentration increases, the absorbance of the synthesized silver nanoparticles increases. The average crystallite size as calculated from the Scherrer's formula is 18.02nm. The average surface charge potential is -12.6 mV showing their stability and the average particle size obtained from DLS analysis is 29.6 nm. These biosynthesized AgNPs have a potential application in antibacterial, antimicrobial, anticarcinogenic, and other medicinal fields.

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Debangana Ghosh

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