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## A concise review on nanomaterials: Plant-based synthesis, optimization and characterization

### ABSTRACT

Numerous studies have revealed that plants' secondary metabolites possess the electrochemical capacity that plays a major role in the reduction of metallic ions to their respective metallic nanoparticles. After the reduction process, these metabolites encircle the fabricated nanoparticles thereby lowering their surface energies and strengthening their repulsive forces, which opens a door to the possibility of achieving promising operational stability in their colloidal system. It is imperative to note that optimization of reaction parameters like pH, concentration, temperature, and time is very crucial when embarking on the green approach to the synthesis of nanoparticles that could be using secondary metabolites from plants as a reducing, capping, and stabilizing agent. This review discussed the role of secondary metabolites and optimum reaction parameters as the most essential and environmentally acceptable requirements for the synthesis of nanoparticles. It also examined the processes underlying their fabrication, purification, and characterization.

**Keywords:** Synthesis, Secondary metabolite, Optimization, Nanoparticles, Stability, Purification, Characterization

### 1. INTRODUCTION

Civilization has encouraged society to take steps to save the ecosystem and preserve resources for future generations. Green nanoscience and nanotechnology aim to produce and improve nontoxic, biocompatible nanomaterials with long-term advantages. Consequently, the sustainability of functionalized nanomaterials is becoming more important than the various applications of nanoscience and nanotechnology, notably in a clinical context, and for this reason, the future development of nanoscience and nanotechnology will be influenced by its ability to "go green." [1]. The goal of green nanoscience and nanotechnology is to fabricate and enhance properties of non-hazardous, and biodegradable nanomaterials with long-term utility. The field of nanotechnology becomes problematic, especially when it comes to

the production of nanomaterials that are not biocompatible and sustainable [2].

### 2. THE CONCEPT OF THE WORD NANO

The term "Nano" originated from the "Greek" meaning "dwarf." It is used as part of a word to describe one-billionth of material and mathematically denoted "1/10<sup>9</sup>". The word "nanometre" abbreviated as nm, means one part per billionth of a meter. Nanomaterials are materials produced within the nano range (1 – 100 nm) and are employed in various fields of endeavours as a result of the contemporary breakthroughs in the disciplines of nanoscience and nanotechnology [3, 4]. This is because metallic nanoparticles and their composites were synthesized and found to have effective measures in electrical and optical sciences [5], scavenging radicals, inhibiting microbial growth, and degrading cancer cell proliferation [6 - 8].

#### 2.1. Different methods for creating metal nanoparticles

Before we move ahead to synthesize nanoparticles, we must understand what is meant by nanosized and within what range a synthesized nanoparticle can have all the requirements of what

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we are looking for in terms of properties. A nanoparticle that has all the properties required for its synthesis purpose is depicted to have a size within the range of "1 – 100 nm". This size can be achieved either by scaling down a bulk particle or assembling atomic particles to form the recommended nanosized [4]. In the synthesis of nanoparticles, both top-down and bottom-up methods

are possible (fig.1). The bottom-up method involves the gathering or joining smaller particles of atomic-sized to generate nanosized structures, whereas, in a top-down strategy, the granular substance is broken down into nanoscale structural elements or fragments. The following diagrams illustrate the above-mentioned strategies [4].

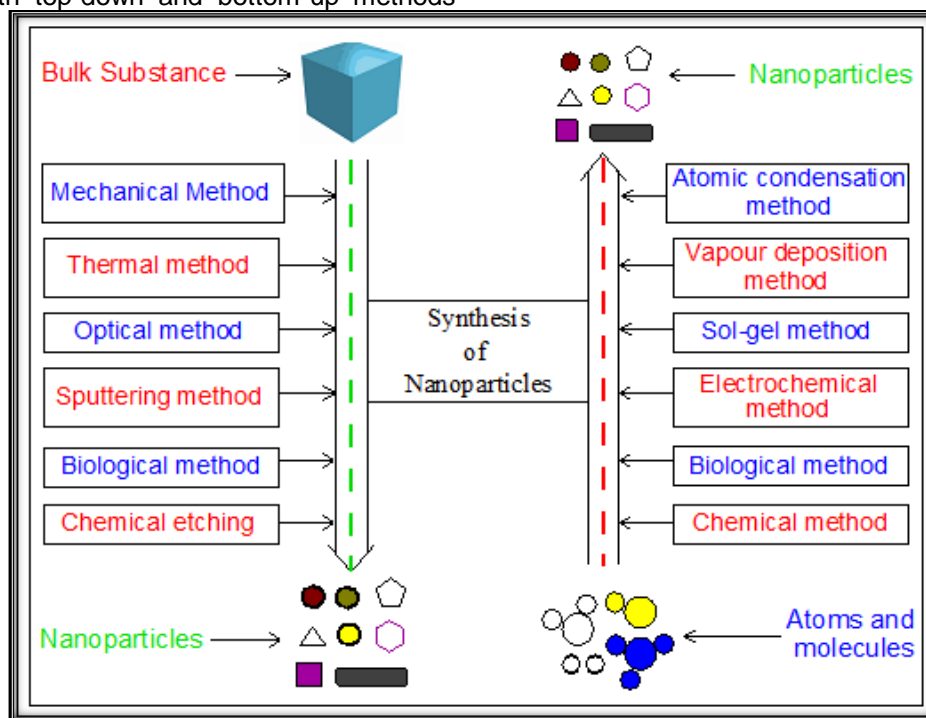


Figure 1. Top-down and bottom-up strategies for the synthesis of nanoparticles

## 2.2. Classification of Nanomaterials

Materials that are produced on the nanoscale can be grouped into two main classes (figure 2) which are further subdivided according to their content and dimensions [4].

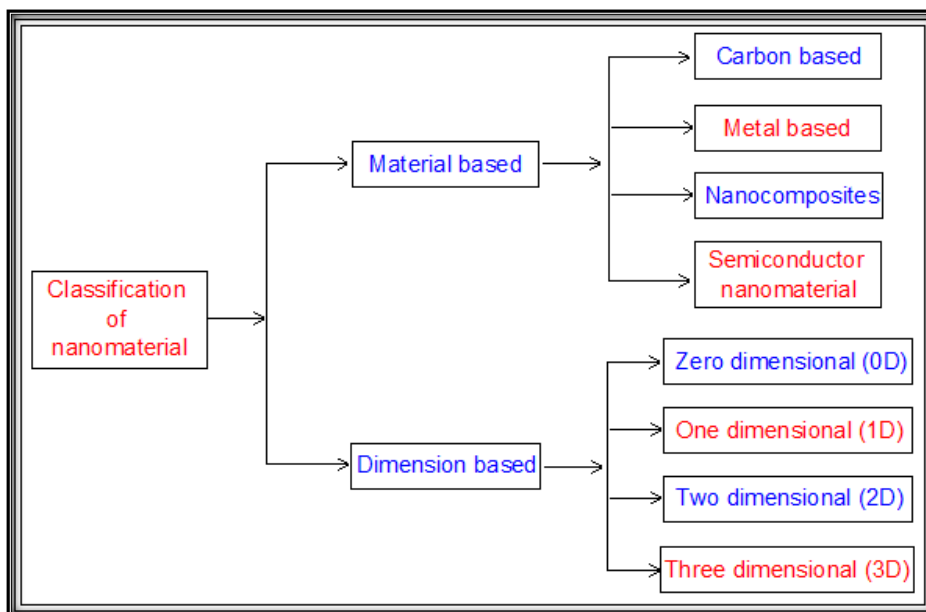


Figure 2. Flow chart for the classification of nanomaterials

### 2.3. Dimension-Based Nanoparticles

Dimension is an important property that has been used to differentiate between different nanostructured modal designs. Nanostructures need to have a minimum of one dimension, ranging from 1 to 100 nm. Figure 3 below provides the greatest explanation of how dimension-based nanostructured materials differed from one another in terms of length, width, height, or depth[9].

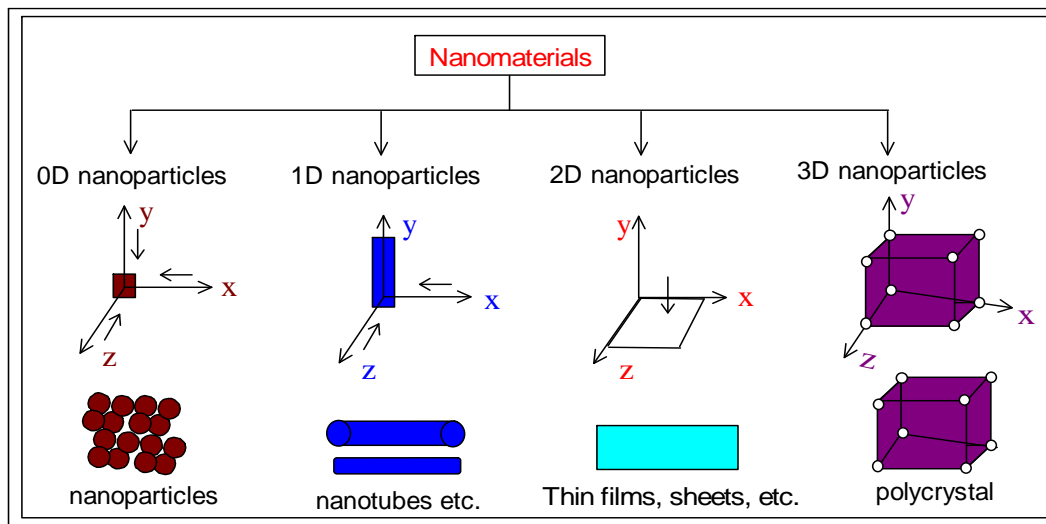


Figure 3. Schematic diagram showing how dimension-based nanomaterials differ from one another

### 3. ROLE OF SECONDARY METABOLITES IN THE PRODUCTION OF NANOPARTICLES

Right now, researchers in the areas of nanoscience and nanotechnology have pointed out that applying the green route technique can make nanoparticle creation easier. This is because it is more affordable, less prone to failure, eco-friendly, and simple to describe[10, 11]. These are the main characteristics that set it apart from more traditional techniques for creating nanoparticles. Notably, the physicochemical and biological properties of the green-synthesized nanoparticles have garnered significant interest from several industries, including biomedical, cellular imaging, medication

delivery, cosmetics, agrochemical, food processing, and food packaging. The green approach to the synthesis of metal/metal oxide nanoparticles uses secondary metabolites from both plants and animals as the essential ingredients that can be used in the synthesis of nanoparticles due to their distinct chemical characteristics and application in pharmacology and industry [12]. A few of these to be mentioned include alkaloids, carbohydrates flavonoids, phenolics, proteins, saponins, tannins, and terpenoids. For synthetic applications, secondary metabolites are crucially considered ideal for many grounds (figure 4).

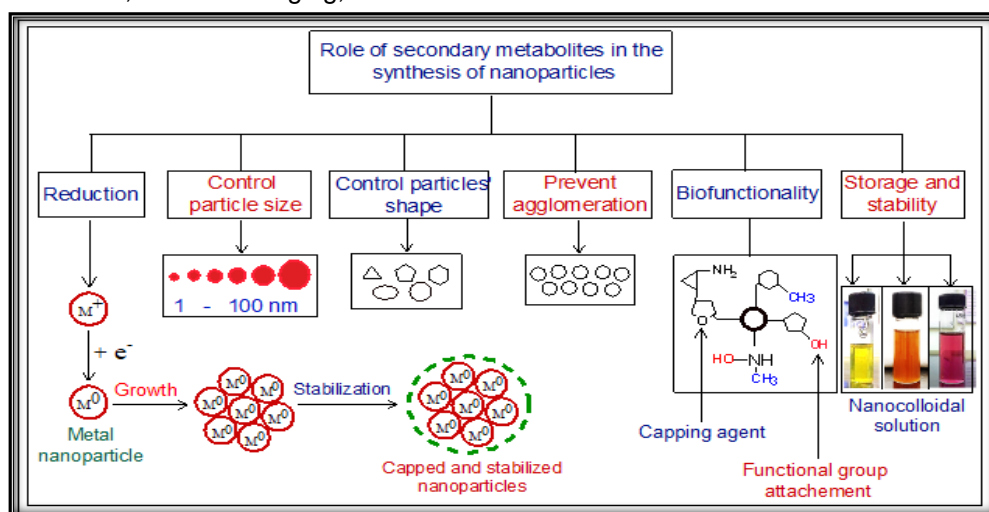


Figure 4. The effects of secondary metabolites in the synthesis of nanoparticles

Additional grounds that are significantly considered as the role of secondary metabolites in the synthesis of nanoparticles and their materials are outlined and discussed below:

### 3.1. Secondary Metabolites as Reduction, capping, and Stabilizing agents

A variety of secondary metabolites have functional units like -OH, -COOH, -NH<sub>2</sub>, -CONH<sub>2</sub>, -OCH<sub>3</sub>, -C=O, -CHO, and -SH that can function as reducing, capping, and stabilizing agents in the creation of nanostructures of different sizes, shapes, and dimensions. They work by creating stable complexes on the nanoparticles' surfaces, which lowers their surface energies and prevents agglomeration thereby inducing operational stability. Capping agents have a crucial role in controlling the size of nanoparticles and enhancing their biological activity, which helps prevent biological diseases [13 - 15].

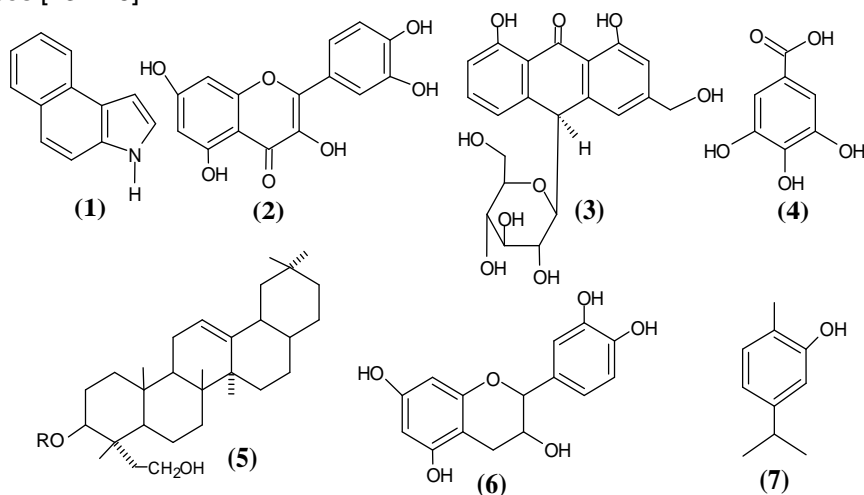


Figure 5. Examples of secondary metabolites with a strong impact on mediating the synthesis of nanoparticles

### 3.3. Increased bio-functionality

Secondary metabolic products can endow extra or additional activity to the nanoparticles. For instance, adding certain secondary metabolites generated from plants to nanoparticle-based systems can enhance their antiradical, anti-bacterial, antifungal, antiviral, anti-inflammation, or antitumor potential.

### 3.4. More ecologically sound and economic fabrication

The green synthetic approach that makes use of the secondary metabolites from plants is more economical, environmentally friendly, and sustainable than the traditional method for creating nanomaterials. This supports the advancement of green nanotechnology and is consistent with the concepts of ecologically conscious chemistry [17].

### 3.2. Bioactivity and Biocompatibility

Many secondary metabolites originating from plants, and animals have these properties by nature, which qualify them for the green preparation of nanoparticles aimed at biomedical uses such as transportation and delivery of drugs to the targeted cell, tissue, and organ or the whole system for therapeutic purposes. The secondary metabolites can also make nanoparticles biocompatible by reducing the possibility of being toxic and enhancing their compatibility with the physiological processes. A few examples of secondary metabolites (fig.5) of this kind include alkaloids (1), flavonoids (2), glycosides (3), phenolics (4), saponins (5), tannins (6), and terpenoids (7), among others (Jain & Mamman, 2023).

### 4. INFLUENCE OF EXTRACT, FRACTION, AND ISOLATED COMPOUND ON GREEN SYNTHESIS

The plants' crude extracts, fractions, and isolated compounds which can mediate the synthesis of nanoparticles can be obtained with the help of protocols illustrated in figure 6 [18 - 24].

### 5. INFLUENCE OF THE REACTION PARAMETERS IN THE SYNTHESIS OF NANOPARTICLES

A process to fabricate nanoparticles uses a variety of techniques and procedures that must be adopted properly so that particles of sizes between 1-100 nm are generated. These sizes endowed them with applicability properties that are distinctively different from their corresponding bulk material and endowed them with various

advantages in the fields of material sciences, electronics, catalysis, agriculture, and medicine, among few. However, a multi-step process involving careful study of numerous elements

(concentration, temperature, pH, and time) must be taken into account to improve yield, homogeneity, and functionality that can be tailored to a particular application.

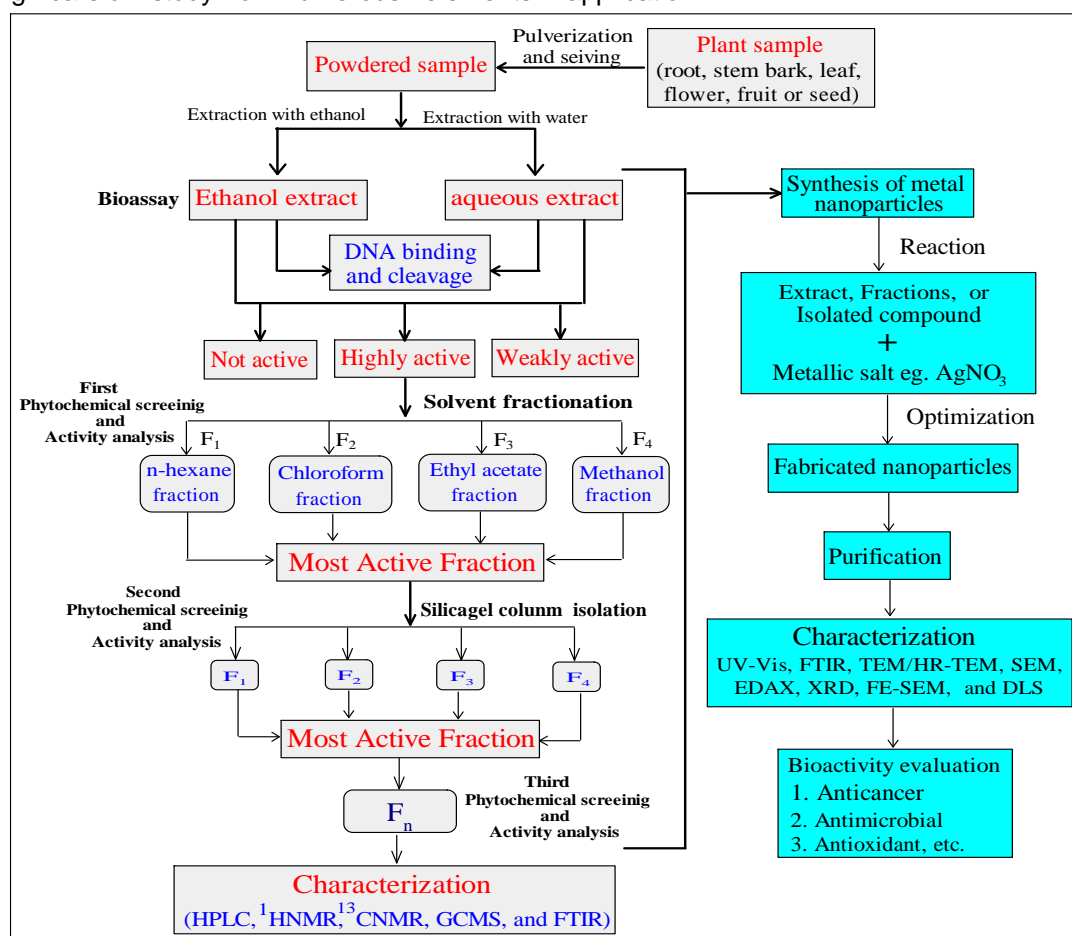


Figure 6. Scheme for green synthesis of nanoparticles, their characterization and biomedical application

### 5.1. Concentration of plant extract, fraction, or isolated compound

The concentration of a plant's secondary metabolite is a significant factor that yields the desired products when embarking on the production of nanoparticles. According to findings from numerous research, this parameter affects the sizes, shapes, and biological activity of the synthesized nanoparticles [25 - 27]. It is essential to understand that, this phenomenon contributes significantly to the stability of the fabricated nanoproducts. Preparation of the variable concentrations of the plants' crude extracts can be achieved by employing a method designed and reported by [28,22]. According to this method, a definite mass (e.g. 20, 30, 40, or 100 mg) of the plant extract (crude, fraction, or isolated compound) will be dissolved in a definite volume of distilled water to yield a standard stock solution. Solutions of lower concentration (figure 7) that can

also be employed when embarking on the synthesis of nanoparticles can be prepared from the stock solution by the use of the serial double dilution method [6].

### 5.2. Concentration of metal ions (e.g., $Ag^+$ , $Au^{2+}$ , $Fe^{2+}$ , $Fe^{3+}$ , $Cu^{2+}$ , $Zn^{2+}$ , etc)

Metal ion concentration is crucial to the biosynthesis of nanoparticles because it controls their formation by monitoring a trend that indicates an increase in metal ion concentration results in an increase in the yield of the desired product (metallic nanoparticles) by cutting down the reaction time [11]. This is particularly crucial if the sample of plant extract in use contains strong and enough phytochemicals that can reduce, cap, and stabilize the metal ions to their corresponding nanoparticles. It is worthwhile to understand that, variations in the concentrations of the ions of the metal (e.g.,  $Fe^{3+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Ag^+$ , or  $Au^{2+}$ ) have a

great impact on the morphology, physicochemical potential, and applicability of the nanoparticles. Particularly, solutions with concentrations in the

range of 0.1 to 2.0 mM; 1.0 to 5.0 mM; or 20.0 to 100.0 mM were employed in several studies [29].

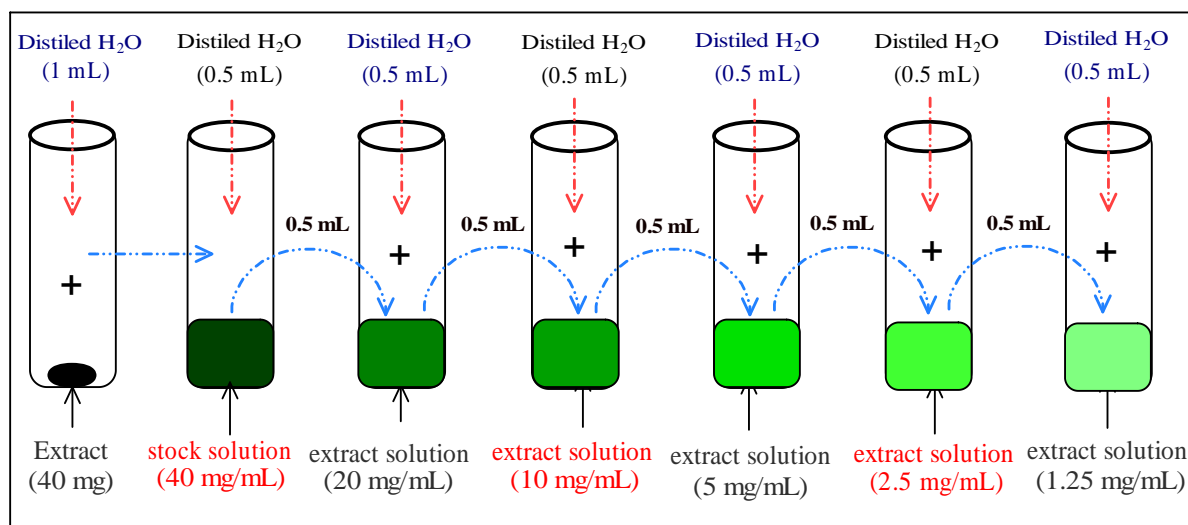


Figure 7. Preparation of stock solution of plant extract, and other working concentrations that can be used to synthesize metal/metal oxide nanoparticles

### 5.3. Temperature

Temperature is one of the parameters that substantially have an impact on the biosynthesis of metallic nanoparticles because it positively influences the reaction rate[30]. The majority of these synthetics were reportedly wrapped up at room temperature, for the fact that secondary metabolites are organic molecules that have lower reaction temperatures, makes it evident that working with them at normal temperature is essential for their stability and applicability. To speed up the process and improve the conditions for the complete conversion of  $\text{Fe}^{3+}$  to  $\text{Fe}^0$ ,  $\text{Cu}^{2+}$  to  $\text{Cu}^0$ ,  $\text{Ag}^+$  to  $\text{Ag}^0$ ,  $\text{Au}^+$  to  $\text{Au}^0$ ,  $\text{Au}^{3+}$  to  $\text{Au}^0$ , and  $\text{Cu}^{2+}$  to  $\text{Cu}^0$ , synthetic experts recommended a working reaction temperature of 40°C, 60°C, and 80°C [31]. The results of the investigation showed that in comparison to nanoparticles made at lower temperatures (313°K and 333°K), those made at higher temperatures (353°K) have better morphologies and intense absorption peak for the surface plasmon resonance which substantially stands for the promising yield of the nanoparticles produced[26, 32, 33].

### 5.4. Influence of pH (Acidity) or pOH (alkalinity)

The degree of acidity or alkalinity of the secondary metabolites has a significant influence on the formation of metallic nanoparticles in a variety of ways, including changing the charge on the secondary metabolite, influencing the electrochemical process that takes place during the reduction reaction, and the interactive binding that

take place between the secondary metabolite and synthesized metal nanoparticles[31]. The yield, dimension, morphology, morphology, size, and stability of the synthesized nanoparticles are also influenced by pH [19, 34].

## 6. BIOSYNTHESIS OF METAL/METAL OXIDE NANOPARTICLES

The first and most important stage to attain when embarking on eco-friendly synthesis (Green synthesis) of metal/metal oxide nanoparticles, is the conversion of a metal ion (e.g.,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Au}^{2+}$ , etc) to neutral metal species ( $\text{Fe}^0$ ,  $\text{Cu}^0$ ,  $\text{Zn}^0$ ,  $\text{Ag}^0$ ,  $\text{Au}^0$ , etc). This aforementioned process is known as reduction which is done by the phytochemical ingredients that make up the plant extract and fractions[35]. They are also responsible for stabilizing, sizing, and shaping the synthesized metallic nanoparticles [3, 36]. Many researchers have reported the Synthesis of nanoparticles using plant extract of unknown concentration, as well as those in which the extract concentrations were known ranging from 1 - 60 mg/ml [25 - 27]. To synthesize nanoparticles with promising medicinal application and lasting operational stability, a definite volume of the plant extract with a definite concentration will be added gradually (drop by drop) to a definite concentration of the metal salt solution by stirring at a constant temperature for a specific period. The change in the initial colour of the reaction precursors when compared to the nanoparticle formed (fig.8) served as an indicator for the formation of metal nanoparticles [37, 38].



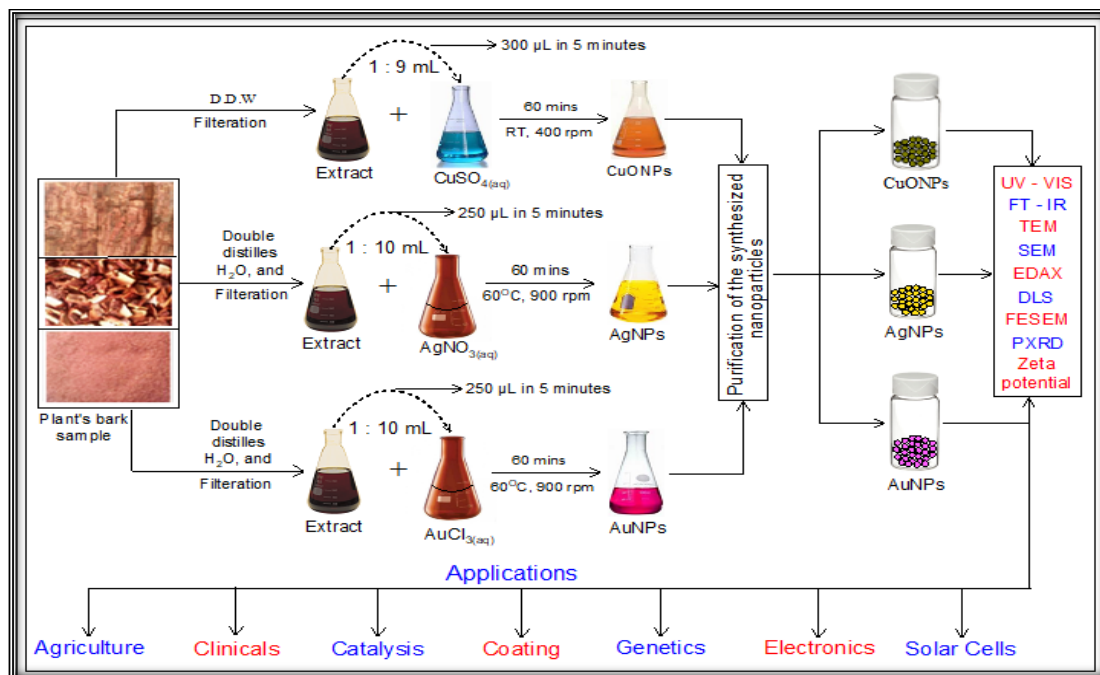


Figure 8. Schematic diagram of plant-based fabrication, characterization, and application of metal/metal oxide nanoparticles

#### 6.1. Mechanism for the green synthesis of metal oxide nanoparticles

Some researchers have proposed a mechanistic pathway based on biochemical constituents of the plant's secondary metabolites that were employed in the green synthesis of metal/metal oxide nanoparticles. They asserted that plant extract cut down the oxidation state of positively charged metal ions to neutral metallic species thereby reducing its surface energy and forming a protective layer, rather than producing a coordinated molecular complex [39, 40]. Following the full reduction of metal ions precursors, reactive

metals like Fe, Cu, Zn, etc reacted with the dissolved oxygen in the solution, thereby forming metal-Oxide nuclei (figure 9). Viewing from another angle, it may be suggested that, after the reduction of the metal ion precursor within the solution of metal precursor, the dissolved oxygen may only surround the reduced and capped metal nanoparticle forming a metal oxide product as can be seen from the following figure (figure 8). This is supported by the higher percentage of elemental oxygen in the analytical EDAX results of every metal oxide nanoparticle.

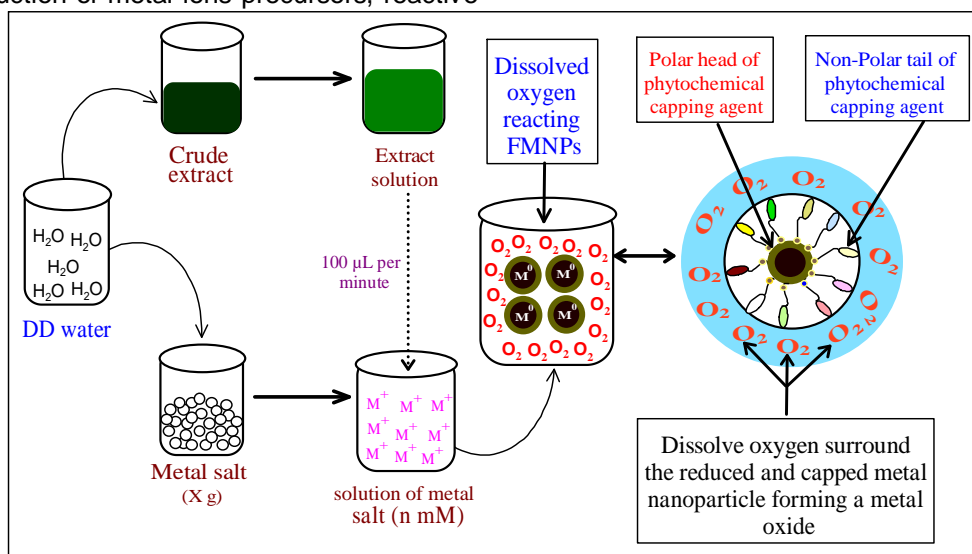


Figure 9. Mechanistic pathways for the synthesis of metal and oxide nanoparticles

Let's not forget that there is more work to be done to precisely understand how plant extract is used as a capping and reducing agent during the manufacture of metal oxide nanoparticles.

## 6.2. Optimization, and SPR Wavelength of Metal Nanoparticles (Ag and Au)

Figure 10 that follows presents the optimization process and SPR wavelength of copper oxide (CuO) and silver (Ag) nanoparticles. The first distinguishing feature that will make anyone understand that metal nanoparticles are formed

during the synthesis of any nanoparticle is the visualization of the colour change between the reactants and those of the finishing products that are free from agglomeration [41]. Depending on their sizes and stability, any optimized and plasmonic nanoparticles must produce a characteristic peak at a particular wavelength due to plasmon resonance [42]. Good examples can be observed with NPs of silver and gold that showed surface plasmon resonance (SPR) peaks within the range of 400 – 500 nm and 500 – 600 nm, respectively [43].

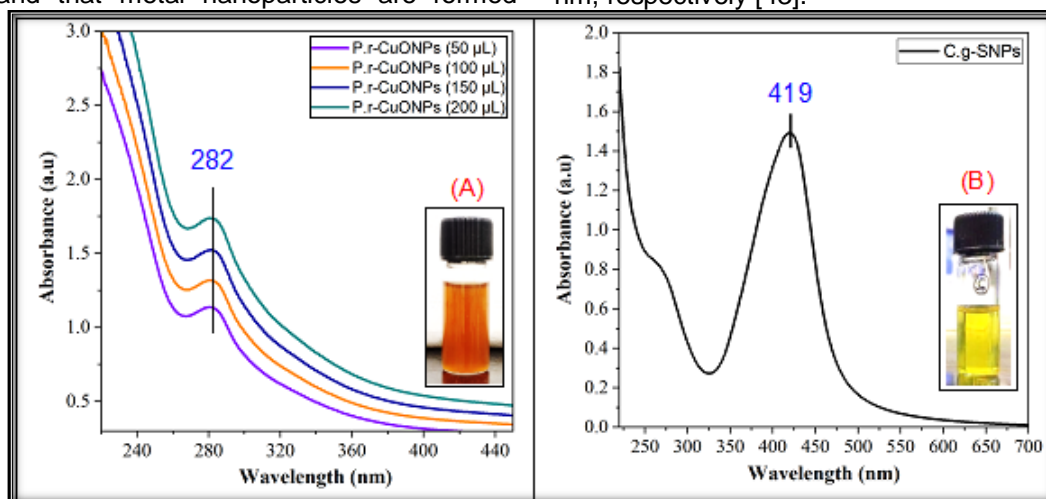


Figure 10. SPR peak of Pr.CuONPs(A) and Cg.AgNPs(B) synthesized at Lab 110 of Sharda University, India

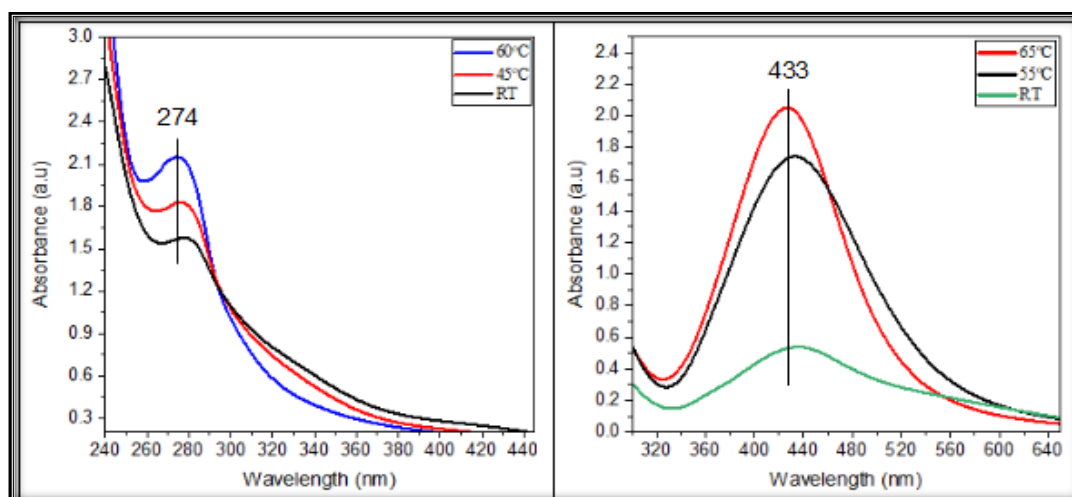


Figure 11. SPR peaks of Pr.CuONPs (274 nm) and Cg.AgNPs (433 nm) synthesized at different temperatures, at Lab 110 of Sharda University, India

As we can see from Figure 11 the surface plasmon resonance of copper oxide NPs appeared exactly at 282 nm and for silver NPs appeared at 419 nm. This phenomenon unveiled smaller sizes compared to those of Figure 10 that appear at 274 nm and 433 nm respectively [44, 45].

Based on this discussion, the CuONPs with SPR absorption peak at 274 nm (figure 11) and AgNPs with SPR peak at 419 nm (figure 10) should be chosen for subsequent production, purification, characterization, and application. This is because robust, stability, and applicability of nanoparticles is a size-dependent phenomenon.



## 7. PURIFICATION OF THE FABRICATED NANOPARTICLES

The rising use of metal/metal oxide nanoparticles has raised human exposure to these particles as well as associated health and environmental problems. This risk is exacerbated when they are used without being purified or separated from the synthesis medium, leaving potentially harmful synthesis precursors not separated from the product and posing a serious risk of needless [46]. The fabricated metal/metal oxide nanoparticles are to be purified through centrifugation for a particular period (e.g., 10, 15 minutes, etc) to get rid of unreacted metal ions ( $M^+$ )

and the phytochemicals that have been used in the reduction of metal ions (figure 12). This is initially done using organic solvent (ethanol or methanol) and finally double distilled water. Nanoparticles that are fabricated and purified have the potential to be stored for a long time without destabilization or any form of aggregation or agglomeration. In normal circumstances, purification is accomplished by taking advantage of the disparities in characteristics between the non-reacted secondary metabolites and targeted nanomaterials. It usually works with three (3) different qualities: particle size, relative polarity, and electrophoretic mobility or solubility.

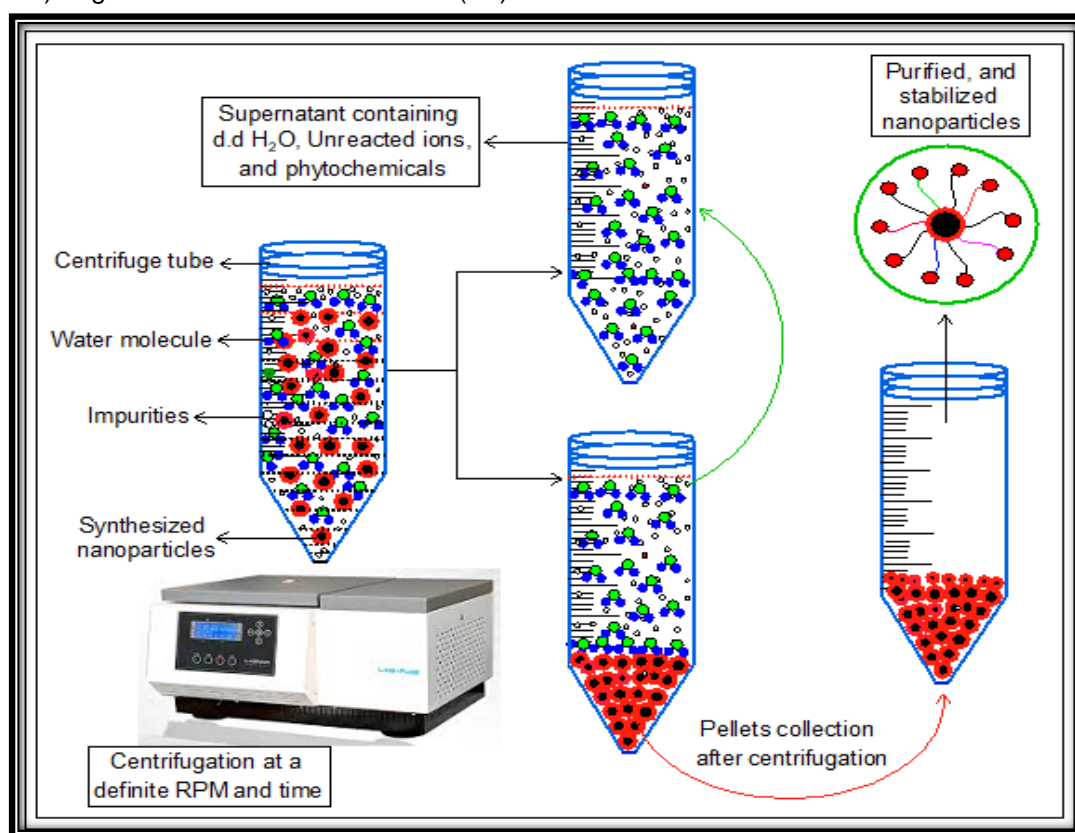


Figure 12. Scheme for the purification of nanoparticles by centrifugation

## 8. CHARACTERIZATION OF THE FABRICATED NANOPARTICLES

The green synthesized and purified metal/metal oxide nanoparticles have been often subjected to characterization using different spectroscopic techniques as mentioned in the below flow chart (figure 13). Many researchers have reported the characterization of different nanomaterials using different analytical tools, but achieved a better characterization, issues like half-way synthesis, short-term stability, and half-way purification should be avoided.

### 8.1. Visual and UV-Vis Characterization of Nanoparticles

The first characteristic that shows you that the nanoparticles you are synthesizing have formed is a visible change in the colour of the reactants to that of the product [47]. Initially, the solutions of the plant extract and metal precursor had definite colours and changed significantly over time when the two reactants were mixed thoroughly and reacted completely with each other. After the formation of the product by observing the characteristic colour change which is distinctively different from the two precursor solutions (plant

extract and metal precursors), UV-Vis studies will be used to obtain surface plasmon resonance (SPR) band at a specific wavelength (nm) for each

plasmonic nanoparticle[43, 48].The below table 1 summarizes the aforementioned information.

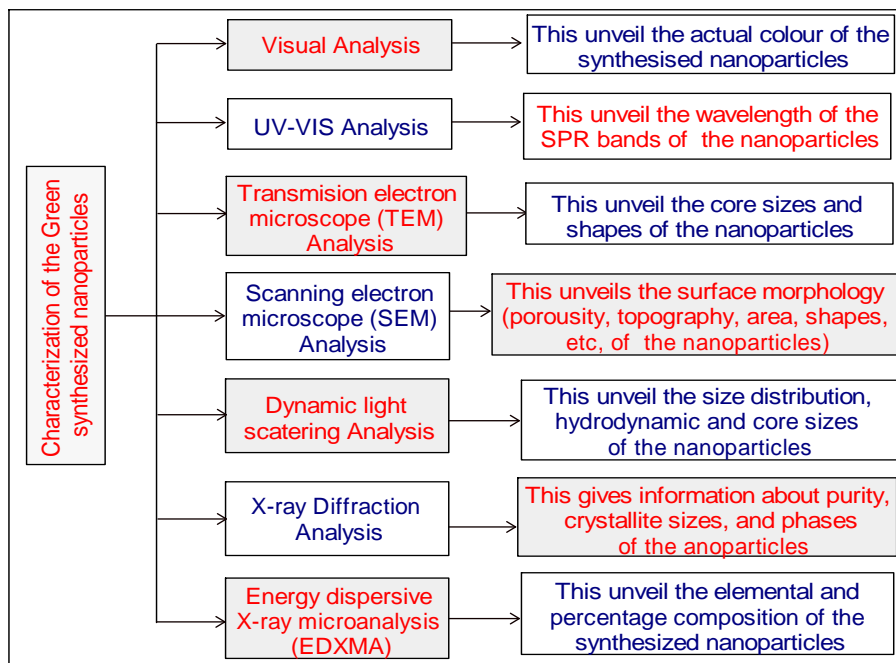


Figure 13. Spectroscopic techniques for the characterization of nanoparticles

Table 1. Plant parts, metal precursors, their product colour, and SPR wavelength

Plant part	Colour of solution	Metal precursor	Colour of solution	Nano Product	Common colour	Wavelength (nm)
Fruit	Black	FeCl <sub>3</sub>	Redish brown	FeONPs	Dark-brown	270 - 300
Seed	Brown	CuSO <sub>4</sub>	Blue	CuONPs	Brown	270 - 300
Flower	Green	ZnSO <sub>4</sub>	Colourless	ZnONPs	Orange-red	350 - 400
Leaf	Orange	AuCl <sub>3</sub>	Yellow	AuNPs	Wine-red	500 - 550
Bark	Red	AgNO <sub>3</sub>	Colourless	AgNPs	Brown/orange	400 - 450
Root	Yellow					
Whole plant	etc					

The interfacial tension of the surrounding medium, size, shape, and purity have a significant impact on the SPR characteristics of plasmonic nanoparticles [49, 50]. Significant shifts in the absorption spectrum may be caused by the high sensitivity of SPR to changes in the medium within which the particles are embedded [3]. For a certain band of wavelengths in the ultraviolet-visible spectrum, statistics are widely available. The principle known as Beer-Lambert is the basis for how the UV-VIS spectrometer works [51]. This aforementioned rule could be described using the figure 14, and its mathematical expression [52].

This aforementioned information and diagrammatic illustration of the law that governs the analysis of a material sample using monochromatic light in the UV region (800 – 400 nm) and VIS region (400 – 200nm) of the electromagnetic radiation give rise to the spectrophotometric data which can be seen in the following spectrum

(fig.15) that we have to acquaint ourselves with. As can be seen categorically from the figure, when an absorption peak changes its position by moving towards the UV region (400 – 200 nm) is referred to as a hypsochromic or blue shift, and if its position changes by moving towards the Vis region (400 – 800 nm) it is referred to as bathochromic or redshift as depicted with blue and red colours respectively (figure 15). The blue shift is characterized by lower energy, lower frequency, longer wavelength, while the redshift is characterized by higher energy, higher frequency, and shorter wavelength. Furthermore, they have no effects on the intensity of the absorption band, which can either be hyperchromic or hypochromic. Hypochromic and hyperchromic shifts are the two phenomena that occur as a function of little or significant changes in the concentration of sample material that has been subjected to UV-VIS analysis.

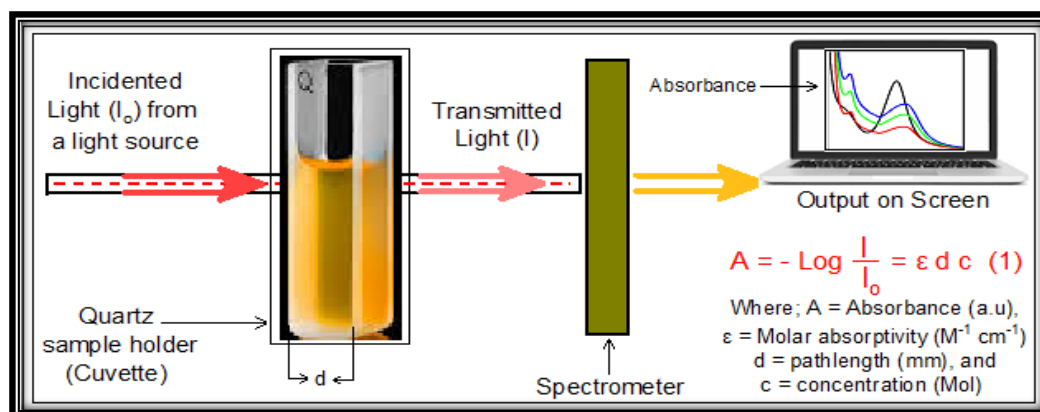


Figure 14. Principle behind the Beer-Lambert law and the basis of UV-VIS spectrometer

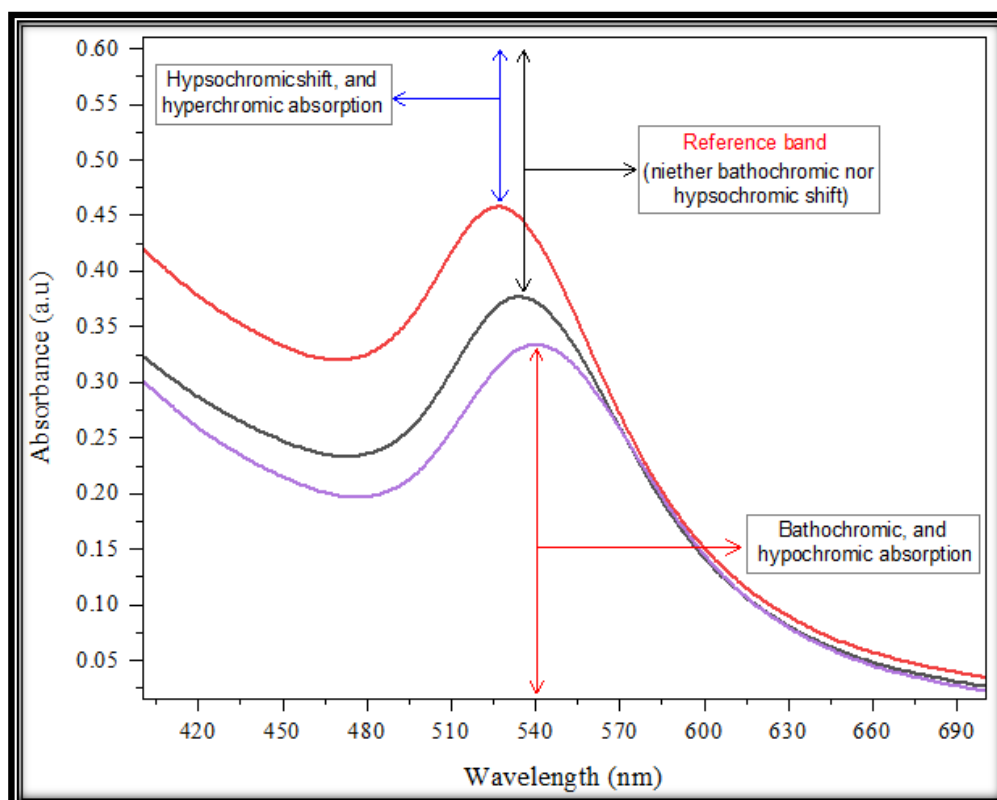


Figure 15. Shift in SPR absorption peaks of AuNPs which occurred as a function of variation in the concentrations, sizes, or shapes

#### 8.1.1. Bathochromic and Hypsochromic shifts as an indicator of variation in size and Molar absorptivity of nanoparticles

Absorption in the UV-Vis region of the electromagnetic spectrum is usually accompanied by two terms commonly referred to as bathochromic and hypsochromic shifts. These shifts rightly communicate a variation in the properties of a test sample to a reference standard. A good example can be seen with UV-VIS absorption peaks of monometallic gold nanoparticles and a bimetallic one that has a silver

shell and a core of gold (figure 16). The effect of the coating or deposition is ascertained by a significant change in the properties of the monometallic nanoparticles in comparison to the fabricated bimetallic counterpart, which is accompanied with a change in properties that may be due to variation in size of the former (precursor) compared to the later (bimetallic product). The hypsochromic shift and rise in molar absorptivity is an indication of the size reduction of the fabricated bimetallic nanoparticles relative to the monometallic precursor which in turn enhances their physicochemical behaviour.

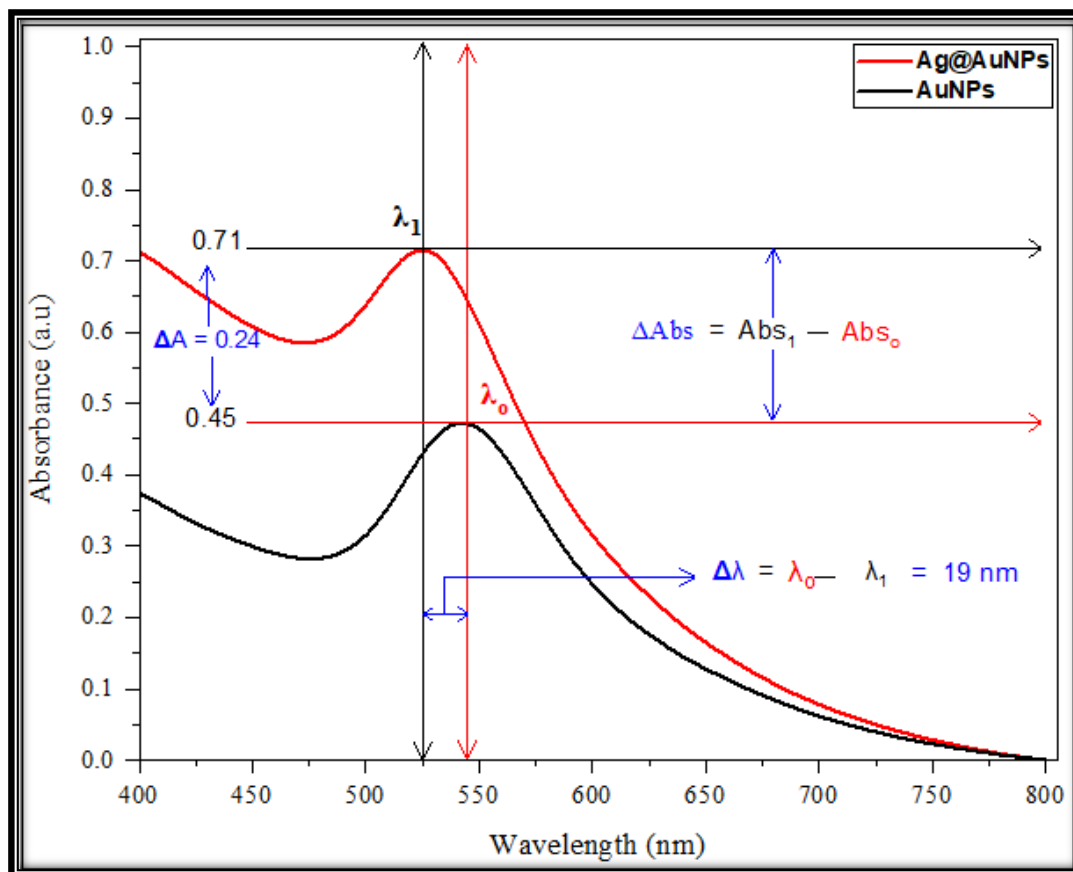


Figure 16. Hypsochromic shift and rise in molar absorptivity of the bimetallic nanoparticles (Ag@Au) in comparison to the monometallic nanoparticles (AuNPs)

## 8.2. Characterization using Transmission Electron Microscope and Scanning Electron Microscopy

For the past 8 decades, electron microscopy has been a ground-breaking imaging technique for research in the field of science and technology, providing access to the world of nanoscale materials and allowing for the characterization of their distinct features. Its ability to scan a particle of submicron-sized up to even individual atoms has led to the emergence of nanoscience and nanotechnology and made it possible to scale down bulk materials to their nanosized components leading to some truly fascinating advancements [53, 54]. The ground-breaking revelation of cathode ray-like electrons in the early 1920s by Louis de Broglie cleared the door for the creation of an electron microscope that used an electron beam to produce a kind of wave motion. With the help of these discoveries, Ernst Ruska and Max Knolls created the first electron microscope in 1931, which they later upgraded into a transmission electron microscope (TEM) in 1933 with the help of the Sieman's company [55]. The disciplines of material science, nanoscience and technology, biology, and other scientific studies all make extensive use of TEM because it produces a magnification/resolution

that is often promising (better than 0.1 nm), which validates its usage as an excellent instrument for probing the structure of materials [56]. It has made a significant contribution to the understanding of the microscopic environment and has been instrumental in some important scientific breakthroughs due to they are capable of using bright-field, dark-field, and high-resolution photography modes, among others. These modes offer various contrasts and highlight numerous structural features in the material being studied. The surface morphology and topography of samples can be seen at high magnification using the potent photographic tool known as scanning electron microscope (SEM). This tool offers thorough data about the composition, texture, and shape of a material sample. In SEM, the surfaces of the sample are interacted with a focused stream beam of electrons, and the resulting impulses are captured as pictures. SEM is superior to other optical modalities in that it can analyze both polar and non-polar samples and has a great resolution of up to the nanoscale scale. It has uses in numerous sectors, notably the electronics sector, biological sciences, forensic analysis, geological sciences, materials research, and nanotechnology [54, 57].

### 8.3. Characterization using X-ray Diffraction

X-ray diffraction (XRD) is a non-destructive spectroscopic method that is frequently used in the areas of material science including chemistry, geology, physics, etc., to probe atomic arrangements, identify crystalline phases and their orientation, measure their thickness, and structural characteristics like lattice parameters, strain, grain size, and epitaxy of a material sample. It operates on the idea of the scattering of X-rays by the atoms in the crystal lattice. When a beam of X-ray is focused on a crystal substance, the X-rays interact with the atoms that make up the crystal and diffract in particular ways (fig.17A and B). This causes the reflected X-rays to generate a pattern of positive and negative interference that may be identified and quantified. By analyzing the angles and intensities of the diffracted X-rays, it is possible to

calculate the crystallite sizes and determine the arrangement of atoms that make up the nanocrystals, the volume of the unit cells, and the purity of the product formed [14]. The signal intensity and broadening from the XRD diffraction spectra (Fig.16C) are two important phenomena that unveiled that, the fabricated nanoparticles are relatively smaller in diameter and conform with previous research[58]. The crystallite sizes of all the green synthesized and biologically capped NPs can be calculated using gathered from the XRD data with the help of the Scherrer equation (eq.1)

$$D = \frac{0.9\lambda}{\beta \cos \theta} \quad (1)$$

D = Crystallite Size (nm),  $\lambda$  = wavelength of the X-ray (nm),  $\beta$  = Full Width Half-Maximum (FWHM), and  $\theta$  = Angle of diffraction (degree).

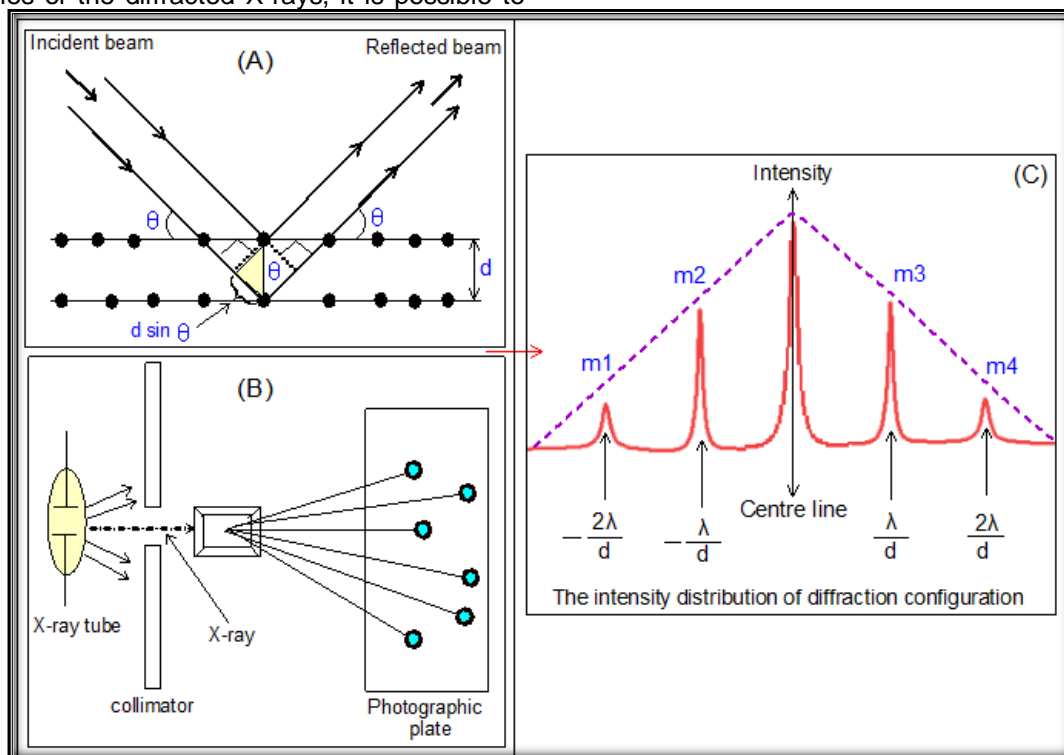


Figure 17. The Principle of XRD and intensity of diffraction configuration

As shown in Figure 17C above, a core peak known as a subsidiary peak on either side of it makes up a diffraction pattern or configuration. The intensity of the secondary maxima declines with increasing distance from the central. The resulting XRD configuration or pattern is typically presented as a graph, a diffractogram, or an X-ray diffraction pattern. By comparing the observed diffraction pattern with known patterns from a database, scientists can identify the crystal structure and composition of the material under investigation.

### 8.4. Characterization using Dynamic Light Scattering Particles Analyser (DLS)

Dynamic light scattering particles analyzer is a spectrophotometric technique that determines the size distribution, hydrodynamic sizes, and core sizes (Fig.18) of particles present in a liquid medium. This technique is frequently referred to as photon correlation spectroscopy. To ascertain the size of the particulates, DLS uses the theory of Brownian motion to measure the movement of particles in a fluid. As such, it is regarded as a non-destructive analytical method that is frequently



employed in different scientific domains like chemical, biological, pharmacological, and material sciences to unveil details that are useful on nanomaterials' sizes and operational stability. It can

be used to examine a variety of systems because it is non-destructive, reasonably quick, and only needs modest sample volumes.

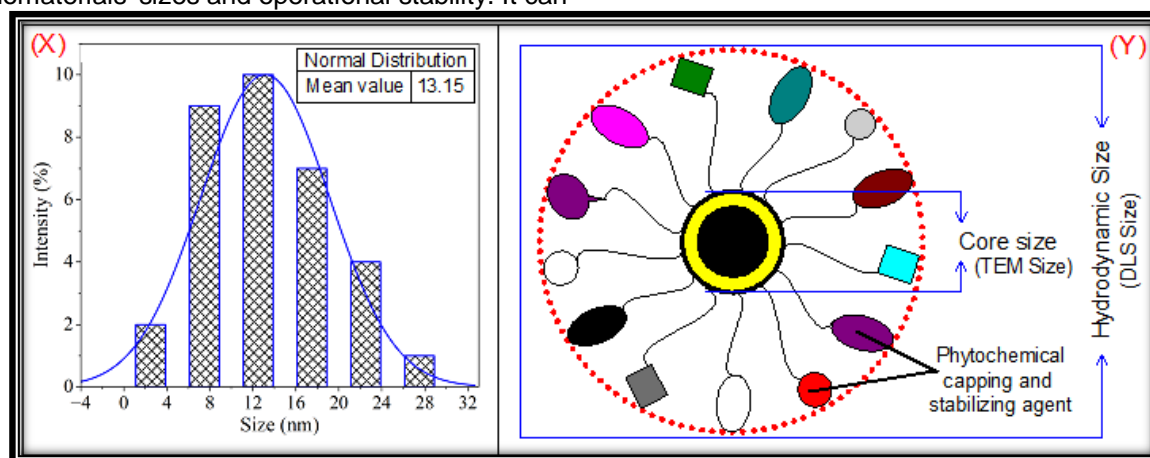


Figure 18. Hydrodynamic Size distributions of green synthesized AgNPs using *Combretum glutinosum* as capping and stabilizing agent

Whenever a beam of laser radiation flows through a liquid mixture, the microscopic particles scatter the light, resulting in oscillations in the intensity of the light that is reflected. Utilizing correlation analysis, the varying intensity of the reflected light is examined. The autocorrelation efficiency, which offers details about the particulate size and dispersion characteristics, is computed using the time-varying oscillations which are utilized to extract the ranges of particles sizes. According to Brownian laws, the light intensity fluctuation of tiny particles in a colloidal system

decay more quickly (figure 19B) than those brought on by larger ones (figure 19A). This decay rate covariance function can be examined to identify the distribution in the sizes of particles within the solution under investigation. Most frequently, a number-weighted or volume-weighted distribution is used to depict the variation in the overall sizes, core sizes, and hydrodynamic sizes of the particles, which is solely dependent on their diffusion coefficient and is connected to their Brownian motion in the solution.

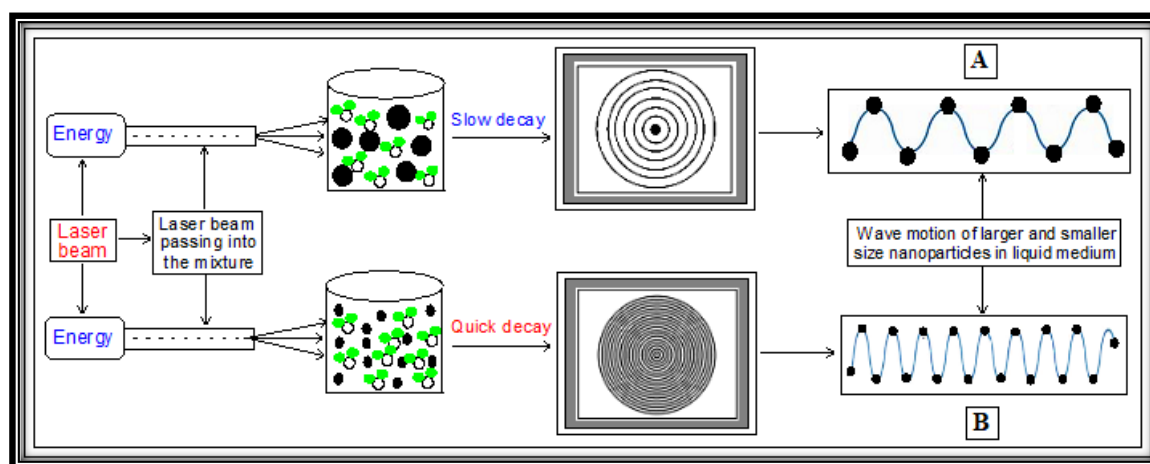


Figure 19. A scheme illustrating diffusion coefficient as a function of Brownian motion of nanoparticles in a solution

#### 8.5. Characterization using Energy Dispersive Analysis of X-Ray

The energy-dispersive X-ray spectrophotometry (figure 20) is an analytical technique that can be used swiftly to detect the presence of elements

within a material and quantify their percentages. It comprises measurement references with well-known structures frequently employed to precisely identify and quantify the elemental compositions that make up a particular sample. It conveniently



does this by comparing the X-ray intensity radiated from the sample with those produced by the referenced compound, which contains predefined amounts of chemical elements. EDX is an electronic device that is made up of an X-ray source, a sample container, and a detector. When using X-ray analysis equipment or specialized electron microscopes, the sample is assaulted with a beam of radiation and that made up the sample is excited by this, which causes them to generate distinctive

X-rays at particular wavelengths which depend on the elemental constituents that made up the sample under study. Detection devices, like lithium-drifted silicon detectors or silicon drift detectors, are used to gather these data and interpret them thereby unveiling the real elemental constituents of the sample, due to a distinct set of X-ray reflective frequencies is connected to every single chemical element in the periodic table.

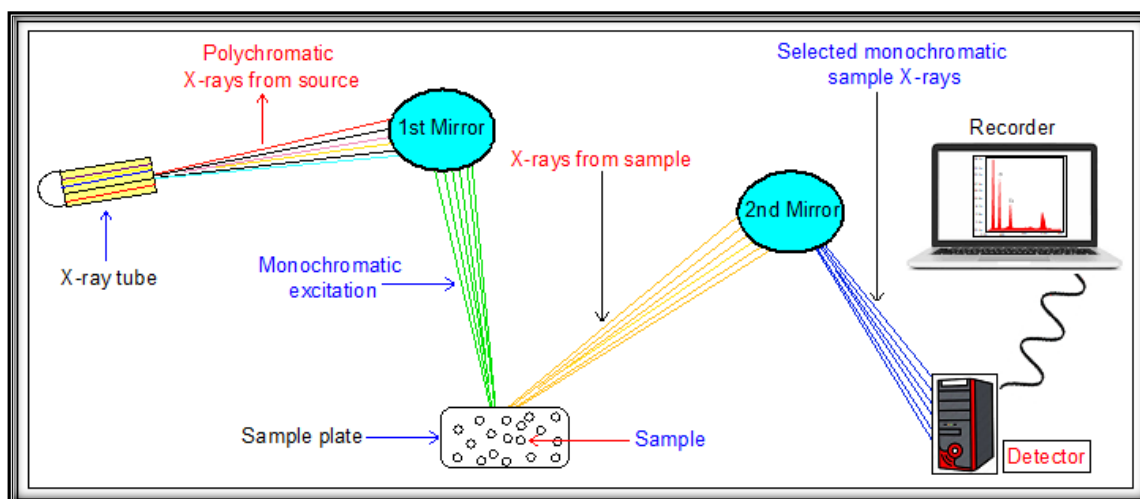


Figure 20. The working principle of energy-dispersive X-ray spectrophotometry

## 9. CONCLUSION

A green synthetic approach to the production of nanoproducts is an environmentally friendly way that depends on low-energy procedures and organically available starting materials. This technique has garnered significant interest in the field of material science due to its environmental friendliness, size-dependant properties, dependability, and sustainability in the synthesis of a diverse array of nanomaterials, such as metal (Ag or Au), metal oxide (FeO, CuO, ZnO, etc), hybrid materials, and bioinspired materials. To enable the purification of nanoparticles, a range of analytical techniques have been devised to isolate them from the raw synthesis material. To recover the reduced, capped, and stabilized metal/metal oxide nanoparticles from crude production and enable their purification, multiple analytical methods have been invented. There is a wide variety of purification and characterization techniques available; the best technique will vary depending on the kind of nanoproducts being produced, and their intended usage. For subsequent uses, it can be essential to preserve the true nature of the synthesized NPs, depending on the purpose. To be more effective and to meet the requirements of environmental sustainability, suitable methods still

need to go through some optimizations. To separate nanoparticles more effectively, it is more crucial than ever to create new techniques or improve ones that have already been employed, while also guaranteeing an affordable, adaptable, and environmentally responsible approach. Since numerous assessments are typically required to identify all relevant features of nanomaterial, characterization shouldn't be restricted to a particular tool.

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### Abbreviations:

AgNPs. Silver Nanoparticles  
AuNPs. Gold Nanoparticles  
C.g. Combretum glutinosum  
CuONPs. Copper Oxide Nanoparticles  
FeO. Iron Oxide Nanoparticles  
P.r. Pterocarpus rotundifolius  
SNPs. Silver Nanoparticles  
ZnO. Zinc Oxide Nanoparticles

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## IZVOD

### SAŽETI PREGLED NANOMATERIJALA: SINTEZA NA BAZI BILJAKA, OPTIMIZACIJA I KARAKTERIZACIJA

Brojne studije su otkrile da sekundarni metaboliti biljaka poseduju elektrohemijski kapacitet koji igra glavnu ulogu u redukciji metalnih jona u njihove odgovarajuće metalne nanočestice. Nakon procesa redukcije, ovi metaboliti okružuju proizvedene nanočestice i na taj način smanjuju njihovu površinsku energiju i jačaju njihove odbojne sile, što otvara vrata mogućnosti postizanja obećavajuće operativne stabilnosti u njihovom koloidnom sistemu. Imperativ je napomenuti da je optimizacija reakcionih parametara kao što su pH, koncentracija, temperatura i vreme veoma ključna kada se krene sa zelenim pristupom sintezi nanočestica koje bi mogle da koriste sekundarne metabolite iz biljaka kao agens za redukciju, zatvaranje i stabilizaciju. U ovom pregledu se raspravljalo o ulozi sekundarnih metabolita i kao najoptimalnijim zahtevima za sintezu u životnoj sredini. nanočestice. Takođe je ispitao procese koji su u osnovi njihove proizvodnje, prečišćavanja i karakterizacije.

**Ključne reči:** sinteza, sekundarni metabolit, optimizacija, nanočestice, stabilnost, prečišćavanje, karakterizacija.

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