A Comparative Study on The Green Synthesis of Silver Nanoparticles using Leaf, Stem and Root Extracts of the Medicinal Plant *Coleus aromaticus*

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Abstract
The green synthesis of silver nanoparticles (AgNPs) using the medicinal plant Coleus aromaticus presents an eco-friendly alternative to conventional methods. This approach utilizes plant extracts rich in bioactive compounds that act as reducing and stabilizing agents, facilitating the formation of AgNPs. The characterization of these nanoparticles is crucial for understanding their properties and potential applications. In this, the efficiency of leaf, stem and root extracts of coleus aromaticus prepared using ethanol as reducing agent was explored.

When the extracts were mixed with silver nitrate solution, formation of nanoparticles occurred indicated by the colour change. The synthesised nanoparticles were characterized using UV-Vis, FT-IR, XRD and FE-SEM.

Keywords: Nanotechnology, AgNPs, Green synthesis, *Coleus aromaticus*, SEM analysis, FTIR analysis, XRD Analysis.

Introduction

The revolution in nanotechnology marks a pivotal moment in scientific history. Nanotechnology involves the creation, modification and imaging of nanostructures ranging in size from 1 to 100 nm. The concept was first introduced by Feynman and this technological advancement has unlocked new possibilities across various sectors including food packaging, animal husbandry, electronics, agriculture, medicine and healthcare and is recognized as a recent industrial development[4]. Silver nanoparticles (AgNPs) have gained significant attention for their wide range of applications in fabrics, keyboards, wound dressings and biomedical devices[18,19]. Due to their unique surface-to-volume ratio, nanosized metallic particles exhibit markedly altered physical, chemical and biological properties, enabling diverse applications[17,22].

Various synthesis techniques have been developed to meet the growing demand for AgNPs. Traditional physical and chemical methods, however, are typically costly and hazardous. In contrast, biologically synthesized AgNPs have demonstrated high yield, solubility and stability[11]. Among the various synthetic approaches, biological methods are regarded as the simplest, fastest, safest, most reliable and environmentally friendly. They can produce nanoparticles with well-defined sizes and shapes under optimal conditions, making them highly suitable for translational research. Ultimately, green chemistry-based production of AgNPs holds substantial promise.

Green synthesis is favoured over conventional methods due to its cost-effectiveness, scalability for large-scale production and the absence of toxic chemicals, high pressure, or elevated temperatures[24,25]. In the synthesis of silver nanoparticles, researchers have employed various plant-derived materials including extracts from leaves, roots, stems, bark, fruits, buds and latex[21].

The process typically involves the simple mixing of plant extracts with a metal salt solution at ambient temperature with nanoparticle formation occurring within minutes. This technique has been widely used to synthesize nanoparticles of silver, gold and other metals[13]. It is well established that factors such as the type and composition of the plant extract, metal salt concentration, pH, temperature and reaction time significantly influence the rate of nanoparticle formation as well as their size, yield and properties. Notably, the concentration of precursor metal salts and polysaccharides affects the size and distribution of AgNPs. Their morphology, which plays a critical role in determining their behaviour, is influenced by complex interactions among molecular, surface and crystalline properties[26].

Physical methods are generally perceived as time-consuming and are limited by stringent conditions such as extreme temperatures or pressures, making them expensive[12]. In contrast, biological methods, particularly those utilizing plant extracts, bacteria and fungi, are considered safer, as they involve significantly fewer toxic reagents and additives. These methods also offer the potential for rapid, cost-effective and large-scale nanoparticle synthesis. Increasing interest has been directed toward the use of biological sources for nanoparticle production, with plant extracts especially recommended for AgNP synthesis. Plant secondary metabolites including enzymes, polysaccharides, alkaloids, tannins, phenols, terpenoids and vitamins are known for their antibacterial properties[8]. Flavonoids and terpenoids are thought to contribute to the stabilization of AgNPs. *Coleus*, a member of the Lamiaceae family, is a notable plant with environmental and ornamental value. It is commonly propagated vegetatively[23]. Cytokinins have been shown to influence directly several aspects of photosynthesis including chloroplast development, chlorophyll synthesis and degradation, enzyme activity and electron transport.

Moreover, cytokinins stimulate the production of important secondary metabolites in *Coleus aromaticus* Benth (L), enhance flavonoid biosynthesis in *Scutellaria alpina* shoot cultures and increase cucurbitacin content in *Citrullus colocynthis*[10]. Among these, kinetin is considered a particularly significant cytokinin with promising applications[25].

Material and Methods

(1) Extraction of *C. aromaticus*: Different parts of the *C. aromaticus* plant (leaf, stem and root) were collected, thoroughly cleaned and ground into a fine powder. The extraction was carried out using a Soxhlet apparatus with ethanol as the solvent and reducing agent.

(2) Synthesis of Silver Nanoparticles (AgNPs): For each plant extract, a 250 mL Erlenmeyer flask containing an aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared. The respective plant extract was added to the solution to initiate the reduction of Ag^+ ions. To prevent pressure build-up, the reaction mixture was continuously heated on a microwave oven turntable at 300 W for 4 minutes to facilitate complete bio reduction. During the reaction, the colour of the mixture changed over a period of up to 30 minutes, progressing from a light hue to yellowish-brown, then to reddish-brown and finally to colloidal brown, indicating nanoparticle formation. Each plant part extract was processed independently. To avoid photoactivation of $AgNO_3$, all reactions were conducted at room temperature in the dark.

(3) Characterization of AgNPs

UV-Visible Spectroscopy: UV-Vis spectral analysis was performed using a Beckman spectrophotometer (Model No. DU-50, Fullerton, CA, USA) with a resolution of 1 nm. The spectra were recorded in the wavelength range of 200-700 nm. To monitor the progress of Ag^+ reduction, 1 mL samples were collected at time intervals of 0, 15, 30, 45 and 60 minutes, as well as at 24 hours.

Fourier Transform Infrared Spectroscopy (FTIR): Dried AgNPs were subjected to FTIR analysis using the potassium bromide (KBr) pellet method in a 1:100 sample-to-KBr ratio. The spectra were recorded using a JASCO FT/IR-6300 spectrometer equipped with a JASCO IRT-7000 Intron Infrared Microscope operating in transmittance mode at a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan).

Scanning Electron Microscopy (SEM): Colloidal AgNPs were centrifuged at 25,900 rpm for 30 minutes. The resulting pellets were redispersed in 0.1 mL of deionized water. The well-mixed pellet was carefully placed on a clean glass coverslip and allowed to air dry. The dried samples were gold-coated using a sputter coater (Model No. JFC-1600, JEOL, Akishima-shi, Japan) and examined under a Scanning electron microscope (ZEISS EVO-MA 10, Oberkochen, Germany). The SEM images included details such as accelerating voltage, magnification and particle size.

X-ray Diffraction (XRD): XRD analysis was performed according to the method described in literature. A PANalytical XPERT-PRO Powder Diffractometer (PANalytical B.V., Almelo, The Netherlands) was used with monochromatic Cu Kα radiation (λ = 1.5406 Å), operated at 45 kV and 30 mA. The diffraction patterns of the silver nano powder were recorded over a 2θ range of 4.01° to 79.99° at room temperature.

Results and Discussion

The synthesis of silver nanoparticles (AgNPs) using plant extracts involves the interaction of silver nitrate ($AgNO_3$) with various bioactive compounds present in the plant. The process typically occurs in three stages: initial ion reduction, cluster formation and subsequent nanoparticle development. Each stage is influenced by factors such as the nature and concentration of the reducing agent, silver nitrate concentration and pH of the medium. Plant macromolecules such as amino acids, proteins, alkaloids, flavonoids, polyphenols, enzymes, tannins, carbohydrates and saponins contain hydroxyl (–OH) groups that play a critical role in both the reduction of Ag^+ ions to elemental silver (Ag^0) and the stabilization of the resulting nanoparticles. The nucleation of silver atoms further promotes the formation of silver nanoparticles.[18]

(1) Synthesis of Silver Nanoparticles from *Coleus aromaticus*: The biosynthesis of AgNPs using *Coleus aromaticus* extract was carried out by interacting $AgNO_3$ with the plant's bioactive constituents. As described earlier, the nanoparticle formation followed a three-stage mechanism: reduction of Ag^+ ions, cluster formation and growth into stable nanoparticles. The biomolecules present in the extract-particularly those with hydroxyl groups-facilitated the reduction and stabilization processes, leading to the effective formation of AgNPs.

(2) Physical Observation: In a typical reaction, 90 mL of 1 mM silver nitrate solution was mixed with 10 mL of *C. aromaticus* extract. A visible change in the solution's colour, from colourless to light yellow, then to reddish brown and finally to a stable colloidal brown, was observed within 4 hours, indicating the formation of silver nanoparticles. This colour transition, occurring over approximately 30 minutes, served as visual confirmation of Ag^+ reduction to Ag^0. The final colloidal brown colour suggested the successful and complete synthesis of AgNPs. The solution was then allowed to cool to room temperature and left undisturbed for 24 hours to ensure full bio reduction and saturation. UV–visible spectrophotometric analysis was later conducted to confirm nanoparticle formation and stability.

Characterization of Ag NPs

(1) UV-Visible Spectroscopy Analysis: The formation of silver nanoparticles (AgNPs) was primarily confirmed through UV–Visible spectroscopy. The appearance of a dark brown colour in the reaction mixture served as a preliminary indicator of AgNP formation. The bio reduction of Ag^+ ions

was monitored by recording the UV–Vis spectra of the solution in the range of 200–2500 nm at 2 nm resolution, using a scanning speed of 1000 nm/min.

Although a colour change to yellowish brown occurred within minutes of adding the plant extract, indicating the initiation of nanoparticle synthesis, the complete reduction process continued over an extended period. Silver colloids derived from the root, stem and leaf extracts were fully formed after 24 hours of reaction time.

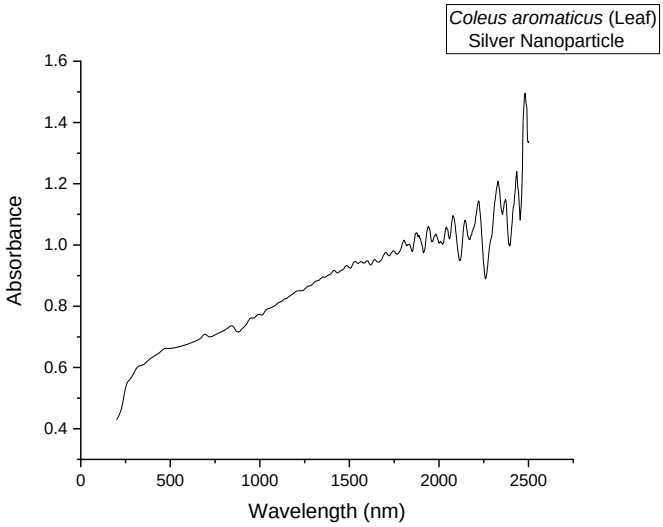

Figure 1(a): UV Analysis of Silver Nanoparticles synthesised from *Coleus aromaticus* Leaf Extract

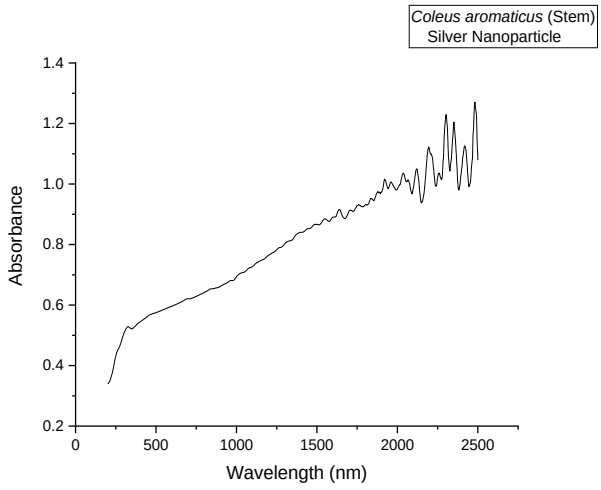

Figure 1(b): UV Analysis of Silver Nanoparticles synthesised from *Coleus aromaticus* Stem Extract

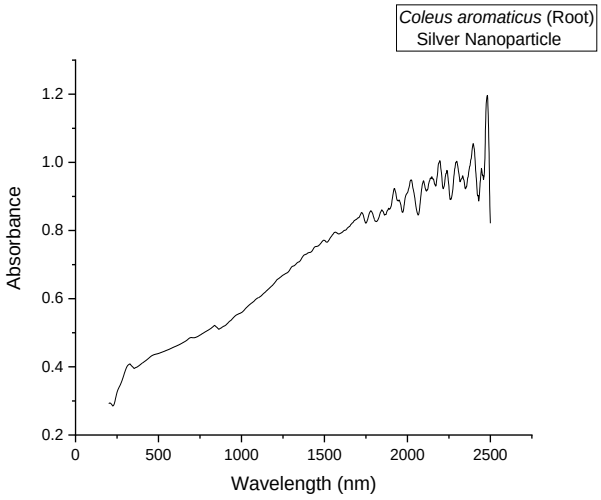

Figure 1(c): UV Analysis of Silver Nanoparticles synthesised from *Coleus aromaticus* Root Extract

The spectral characteristics of the AgNPs provided insights into particle size. A broad peak at a higher wavelength typically suggests the formation of larger or aggregated nanoparticles whereas a sharp, narrow peak at a lower wavelength indicates smaller, well-dispersed particles.

(2) FTIR Analysis: Fourier-transform infrared (FTIR) spectroscopy was employed to identify the biomolecules responsible for reducing Ag^+ ions and stabilizing the synthesized silver nanoparticles (AgNPs), as previously reported. This qualitative technique is based on the detection of characteristic chemical bonds by scanning the sample with infrared radiation. FTIR enables the identification of functional groups in the formation and stabilization of AgNPs. FTIR analysis was performed on three biosynthesised AgNP samples derived from the leaves, stems and roots of *Coleus aromaticus*. The observed absorption bands and their corresponding functional groups are as follows:

Leaf Extract-based AgNPs:
3369.94 cm^{-1} – O–H stretching vibrations, indicating the presence of alcohols and phenols.
1754.02 cm^{-1} – C=C stretching vibrations, indicative of alkanes.
1553.88 cm^{-1} – N–O asymmetric stretching, characteristic of nitro compounds.
1266.88 cm^{-1} – C–O stretching vibrations, indicating the presence of alcohols, carboxylic acids, esters and ethers.
1028.67 cm^{-1} – C–O vibration of hydroxyl groups in alcohols.
800.19 cm^{-1} – N–H stretching vibrations, indicating the presence of primary and secondary amines.

Stem Extract-based AgNPs:
3345.23 cm^{-1} – O–H stretching, associated with carbohydrates, proteins and polyphenols.
1754.02 cm^{-1} – C=C stretching vibrations, presence of alkanes.
1559.10 cm^{-1} – N–O asymmetric stretching, indicative of nitro compounds.
1270.07 cm^{-1} – C–N stretching (amide III band).
808.65 cm^{-1} – N–H stretching vibrations, presence of primary and secondary amines.

Root Extract-based AgNPs:
3362.53 cm^{-1} – O–H stretching, associated with carbohydrates, proteins and polyphenols.
1756.49 cm^{-1} – C=C stretching vibrations, indicative of alkanes.
1576.12 cm^{-1} – N–O asymmetric stretching vibrations, presence of nitro compounds.
1281.92 cm^{-1} – C–N stretching (amide III band).
800.44 cm^{-1} and 731.81 cm^{-1} – N–H stretching vibrations, confirming the presence of primary and secondary amines.

Alkanes, ketones, amines and other functional groups exhibit characteristic absorption peaks in the infrared region, allowing the identification of biomolecules. The absorption spectrum reveals distinct peaks corresponding to specific chemical bonds, reflecting their relative concentrations[20]. By comparing the spectra of the *C. aromaticus* extracts with those of the biosynthesized AgNPs, it is possible to determine the functional groups involved in surface coating and stabilization[2]. Hence, it proves to be a valuable and cost-effective analytical tool for elucidating the role of plant-derived biomolecules in the green synthesis and stabilization of silver nanoparticles.

(3) SEM Analysis: Scanning electron microscopy (SEM) provides valuable insight into the surface morphology, size distribution and aggregation of nanoparticles. Field Emission SEM (FESEM) utilizes a strong electric field to accelerate electrons which then pass through electromagnetic lenses and coils to produce a focused beam that interacts with the sample surface, generating secondary electrons that are detected to reconstruct a high-resolution image.[6] This was conducted for AgNP synthesised using extracts from the leaf, stem and root of *Coleus aromaticus* and the observation are as follows:

Leaf-derived AgNPs:
76.31 nm, 83.94 nm, 91.57 nm, 100.4 nm and 109.3 nm

Stem-derived AgNPs:
48.64 nm, 53.14 nm, 77.26 nm, 78.43 nm, 79.82 nm and 82.54 nm

Root-derived AgNPs:
109.2 nm, 145.9 nm, 147.6 nm, 163.0 nm, 194.4 nm and 212.8 nm

The SEM images revealed variations in particle size and shape across the different plant parts. Metal nanoparticles such as silver, exhibit high electrical conductivity which facilitates SEM imaging without the need for extensive sample preparation.[5] In this study, the samples were coated with gold to enhance conductivity and image clarity. Although SEM does not provide internal structural information, it is highly effective for assessing particle purity, distribution and surface aggregation[16]. The AgNPs synthesised here exhibited morphological diversity including pebble-like, spherical, oval and irregular shapes, consistent with previous reports on plant-based nanoparticle synthesis.[1,3,7,9,14]

(4) XRD Analysis: X-Ray diffraction method was used for structural analysis of synthesised AgNPs using X-ray powder diffractometer (Bruker D8 advance, Germany). The pattern was recorded by CuKα radiation with λ of 1.5406Å and nickel monochromator and ceramic X-ray tube. The scanning was done in the region of 2θ from 10°-90° at 0.02°/min and the time constant was 2s.

The crystalline domain size nanoparticles were calculated through the Scherrer's equation:

$D = K\lambda/\beta_{cos\theta}$

where D = average crystalline domain size, B = Full width half maximum, K = 94, λ = wavelength of X-ray (1.54Å) and θ = Bragg's angle. This method was used for the investigation of crystalline structure of synthesis of AgNPs. The nanoparticles show intense peaks corresponding to (111), (200), (220) and (311).

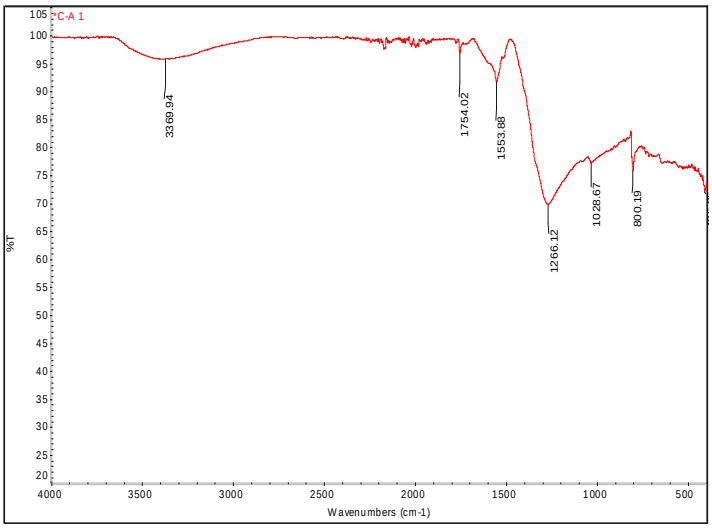

Figure 2(a): FTIR Spectrum of Silver Nanoparticles synthesised from *Coleus aromaticus* Leaf Extract

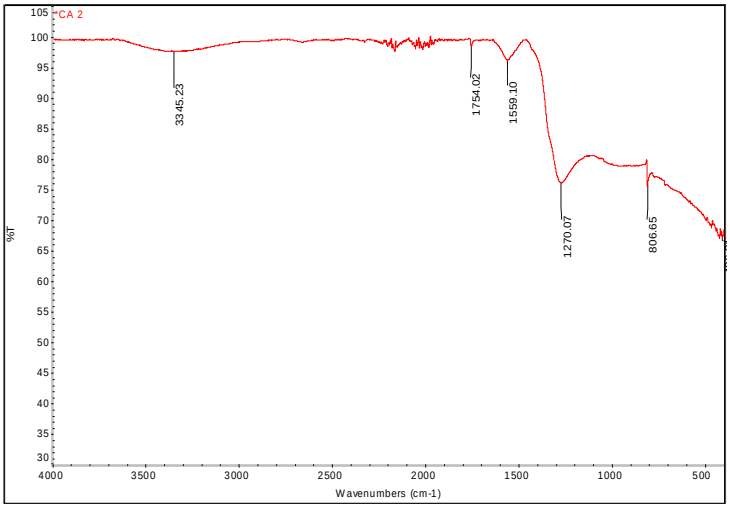

Figure 2(b): FTIR Spectrum of Silver Nanoparticles synthesised from *Coleus aromaticus* Stem Extract

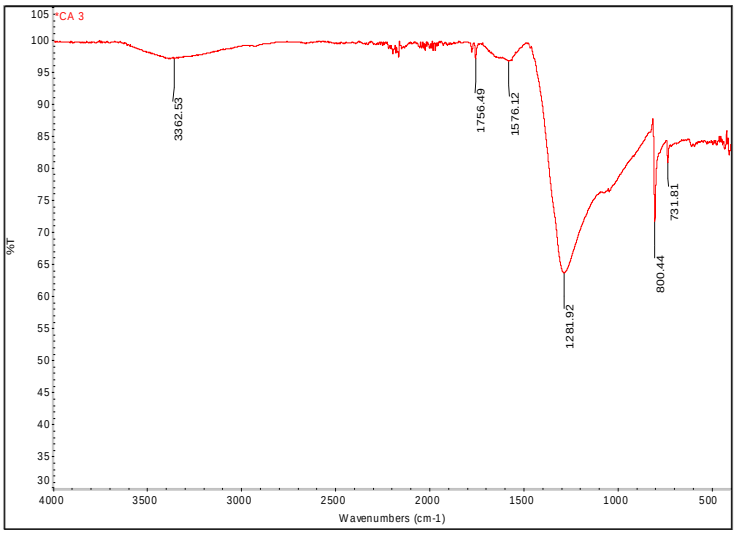

Figure 2(c): FTIR Spectrum of Silver Nanoparticles synthesised from *Coleus aromaticus* Root Extract

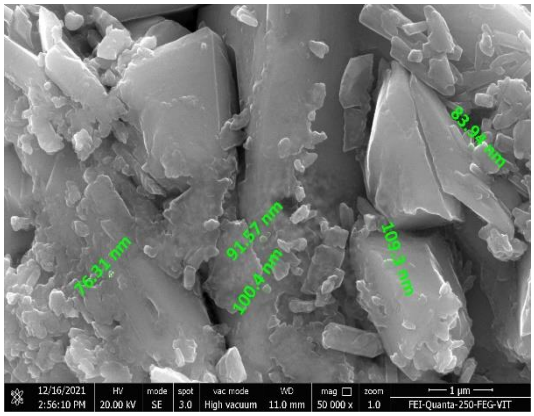

Figure 3(a): SEM Analysis of Silver Nanoparticles synthesized using *Coleus aromaticus* Leaf Extract

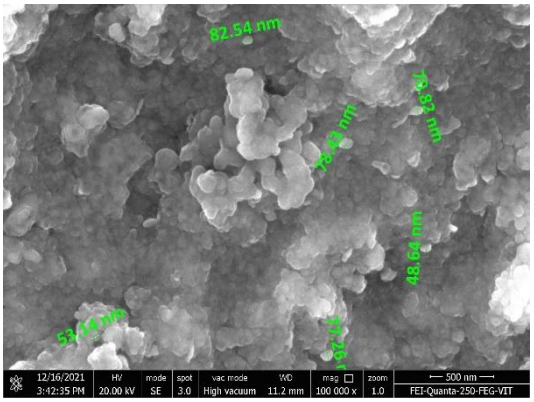

Figure 3(b): SEM Analysis of Silver Nanoparticles synthesized using *Coleus aromaticus* Stem Extract

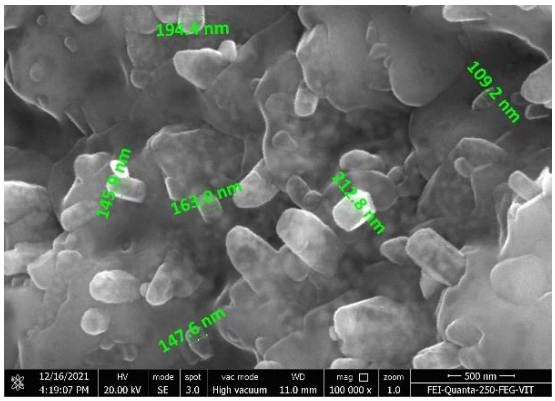

Figure 3(c): SEM Analysis of Silver Nanoparticles synthesised using *Coleus aromaticus* Root Extract

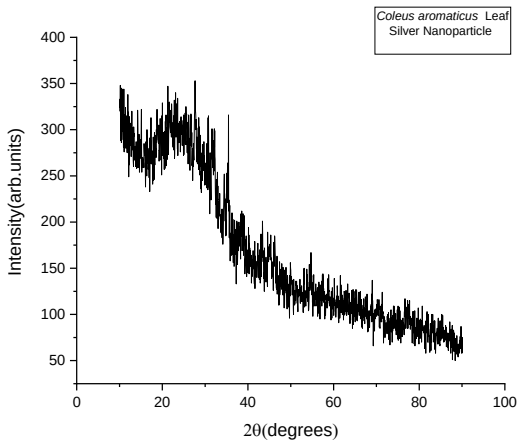

Figure 4(a): XRD Analysis of Silver Nanoparticles synthesised using *Coleus aromaticus* Leaf Extract

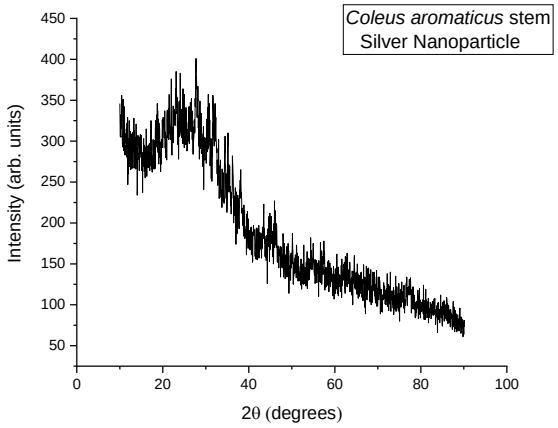

Figure 4(b): XRD Analysis of Silver Nanoparticles synthesised using *Coleus aromaticus* Stem Extract

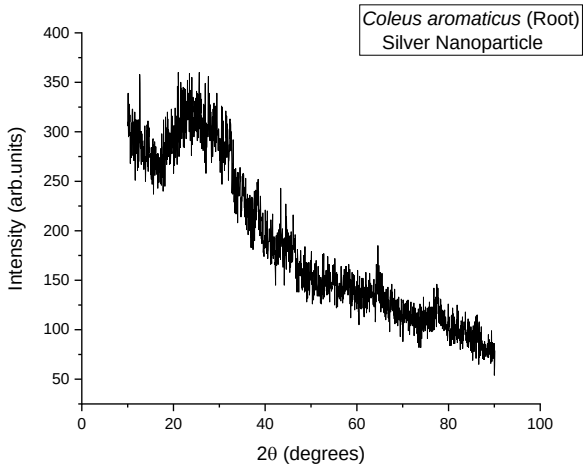

Figure 4(c): XRD Analysis of Silver Nanoparticles synthesised using *Coleus aromaticus* Root Extract

Bragg's reflection based on Face-Centered Cubic (FCC) structure of silver was in good agreement with JCPDS. On calculating the size of AgNPs, the mean size was found to be 100 nm which was in good agreement with nanoparticles size estimated with FE-SEM. The aggregate stack of peaks may be due to the presence of other biological components in the extract.

Conclusion

The metal nanoparticles synthesis using *Coleus aromaticus* extract of leaf, stem and root is simple, efficient and eco-friendly. However, impacts of temperature and pH are yet to be discovered. The advantage is that all parts of the plants are used. Disadvantages may be the size of the nanoparticle produced by root and stem which are more than 100 nm, where the applications are limited. The development of green nanosized materials is an alternative to hazardous chemical synthetic route. An emerging and fascinating area of nanotechnology is the use of plants to synthesise AgNPs, which can influence the development of nanotechnology.

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