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BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACTS OF *CHROMOLAENA ODORATA, TITHONIA DIVERSIFOLIA* AND *SOLENOSTEMON MONOSTACHYUS*

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ABSTRACT
Silver nanoparticles (AgNPs) are highly valued across various scientific fields for their diverse applications in daily life. Sustainable and eco-friendly methods are essential for their synthesis. This study demostrated an alternative, safe, cost-effective and environmentally-friendly approach by utilising leaf extracts from *Chromolaena odorata* (CO), *Tithonia diversifolia* (TD) and *Solenostemon monostachyus* (SM) to biogenically synthesise AgNPs. The resulting colours indicating AgNP synthesis were brown, yellow and brown, respectively. Characterisation of the AgNPs was conducted using UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM). UV-Vis spectra exhibited strong absorption peaks at 445 nm, 437 nm and 430 nm for AgNPs synthesised with CO, TD and SM leaf extracts, respectively. The synthesised AgNPs displayed predominantly spherical morphology, with some triangular and rod-like shapes observed in CO AgNPs, averaging 27.48 nm, 49.64 nm, and 25.76nm in size for CO, TD and SM, respectively. FTIR analysis revealed absorption bands at specific wavenumbers, indicating the importance of carbonyl stretch of amides and O–H functional group vibrations in the bio-reduction, capping and stabilisation of AgNPs.

Key words: Silver nitrate; Silver nanoparticles; Eco-friendly; UV-Vis; FTIR; TEM
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INTRODUCTION
The large category of materials known as nanoparticles (NPs) includes particulate compounds having at least one dimension less than 100 nm (Roy *et al.*, 2013). The huge surface-area of nanoparticles makes them special; nonetheless, the small bulk of the material also makes contributions (News Medical, 2015). Human exposure to nanoparticles has existed throughout history and increased dramatically during the industrial revolution, so that their study is not new (National Nanotechnology Initiative, 2011). For instance, Mesopotamian artisans used nanoparticles to give pot surfaces a glittering outlook as early as the ninth century (Khan, 2016), and Roman glass is the most well-known use of metallic nanoparticles in antiquity. The Lycurgus cups are a form of colour-changing dichroic glass cup from the fourth century AD. The stained glass window, which was frequently used in European churches between the sixth and fifteenth centuries and is still in use now, is another historical example. The vivid colours of these windows are a result of nanoscale gold chloride and other metal oxide and chloride particles. Silver, copper, or other metallic nanoparticles were found to be present in the ceramic glazes used between the ninth and seventeenth centuries in the Islamic world and later in Europe (Tolochko, 2009).

Based on their chemical make-up, nanomaterials can be roughly classified into two groups: organic and inorganic. In contrast to organic nanomaterials, which are made of carbon, inorganic nanomaterials are made of magnetic minerals like gold, platinum, silver, iron oxide, titanium dioxide and zinc oxide. Nanoparticles are increasingly being recommended for use in a number of applications due to their distinctive and novel sizedependent properties. Examples of typical applications include biosensors, catalysts, environmental clean-up, immune-assay labeling, tumour hyperthermia treatment, anti-microbial medicines and vector delivery of therapeutic pharmaceuticals for cancer therapies. The features of synthesised nanoparticles are, however, significantly influenced by a number of variables, including particle size, shape and size distribution, as has been shown by research. Therefore, the control of particle-size and form is essential throughout the manufacturing process. It is possible to alter nanoparticles for specific uses as seen in great manufacturing control (Njagi *et al.*, 2011; Wen *et al.,* 2011; Fu *et al.*, 2013; Mahdavi *et al.*, 2013).

According to Larue *et al.* (2014), Silver nanoparticles (Ag NPs) are the most commercialised nanomaterials among all nanoparticles, and their development from the field of nanotechnology has attracted a lot of interest due to their unique qualities, including chemical stability, good conductivity, catalytic, and most importantly anti-bacterial, anti-viral, anti-fungal as well as anti-inflammatory activities, which can be incorporated into composite fibers, cryogenic sleeves and other materials (Ahmad *et al*., 2003; Logeswari *et al*., 2015). The production of nanoparticles often entails a number of costly, potentially damaging chemical and physical processes that use hazardous and poisonous substances that pose a variety of biological risks. The development of experimental methods for the synthesis of nanoparticles that are biologically inspired is a significant field in nanotechnology.

Creating nanoparticles often entails a number of time-consuming, costly and, perhaps, environmentally-hazardous chemical and physical processes involving the use of hazardous and poisonous substances that pose a number of biological risks. The development of biologically inspired experimental techniques for the production of nanoparticles is a significant topic in nanotechnology (Ahmad *et al*., 2003). To avoid the production of unwanted or harmful byproducts, dependable, long-lasting and environmentally-friendly synthesis procedures must be developed. Utilising the best solvent systems and natural resources, such as organic system, is essential for accomplishing this.

Green technology has been used to create metal nanoparticles that can tolerate various biological elements (e.g., bacteria, fungi, algae and plant extracts). Comparatively to the known green methods of synthesis for metal/metal oxide nanoparticles, which involve the use of bacteria and/or fungi using plant extracts is a comparatively simple and easy method to produce nanoparticles on a large scale. All of these substances are referred to as biogenic nanoparticles (Singh *et al*., 2018a). The availability of plant material, simplicity, efficiency, cheap cost, ease of scalability for mass production, secondary metabolites, purgative qualities, as well as a perfect replacement for traditional nanoparticle preparation procedures are all advantages of using plants as a source for synthesis. Very stable and well-characterised NPs can be produced from the synthesis of NPs when biological components are used properly and effectively. Many researchers have employed green synthesis techniques to produce the particles utilising plant leaf extracts in order to better explore the myriad applications of metal/metal oxide nanoparticles (Singh *et al*., 2018b; Patil and Chandrasekaran, 2020).

The current research has concentrated on producing nanomaterials using nanotechnology-based procedures that support the ideas of green chemistry and minimise or completely eliminate the usage of dangerous chemicals. As a result, environmentally friendly green nanotechnology-based technologies for the production of nanoparticles have drawn significant interest globally (Thakkar *et al.*, 2010). To demonstrate this alternative approach, the study has concentrated on employing biological entities, such as various plant species, to create silver nanoparticles. The plants used were C*hromolaena odorata* (L.) R. M. King & H. Robison, *Solenostemon monostachyus* (P. Beauv.) Briq. and *Tithonia diversifolia* (Hemsl.) A. Gray.

MATERIALS AND METHODS

Chemicals and Plant Material Collection

Without further purification, the analytical-grade reagent that was purchased was employed. Distilled water was used to prepare aqueous solutions in all the experiments. In the vicinity of the Faculty of Life Sciences building at the University of Ilorin, fresh leaves of *Chromolaena odorata* (CO), *Tithonia diversifolia* (TD) and *Solenostemon monostachyus* (SM) were collected.

Preparation of Leaf Extract

The following steps were taken to prepare aqueous leaf extracts: collected leaves were washed with distilled water to clean the surface. The fresh leaves were cut into small pieces, and 0.1 g was weighed and dispensed into a beaker with 10 ml of distilled water. Then, the mixture was heated in a water-bath for 60 minutes at 60° C, to obtain extract while stirring occasionally and then allowed to cool at room temperature. The extracts were filtered using the Whatman No 1 filter paper. The extract was used to synthesise Ag nanoparticles from $AgNO_3$ precursor solution.

Biogenic Synthesis of Silver Nanoparticles

AgNPs were synthesised by introducing 0.034 g of $AgNO_3$ into 200 ml of distilled water. One (1) ml of the extract was introduced into 40 ml of 1 mM of $AgNO_3$ solution and kept in room temperature (Lateef *et al.*, 2015). Subsequently, the synthesis of silver nanoparticles was initially identified by colour change and further analysed by using UV-Vis spectrophotometer, Fourier-Transform Infrared (FTIR) spectrophotometer and Transmission Electron Microscopy (TEM).

UV–Visible absorbance spectroscopy

UV-visible spectrum was scanned with a VWR UV– 6300pc Double Beam Spectrophotometer. The UV-visible spectrum provides information on the actual formation of the metal nanoparticles by surface plasmon resonance effect.

Fourier-Transform infrared spectroscopy

FTIR analysis was done for the determination of functional groups responsible for the formation of Ag nanoparticles; that was actualised using Thermo Scientific Nicolet iS5 FT-IR Spectrometer.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) study was carried out using a model JEM-ARM200F-G TEM. This method uses an electron beam to interact with the ultrathin sample and offers the most precise and high-resolution imaging data regarding the size, shape, morphology, state of aggregation and distribution of nanoparticles at nanometer resolution (Lin *et al.*, 2014; Zhang *et al*., 2016).

RESULTS AND DISCUSSION

Visual Observation

The formation of silver nanoparticles can be easily identified through a visual observation of a distinct colour change. In the case of leaf extracts from *Tithonia diversifolia* (TD) treated with $AgNO_3$, the colour transformation from colourless to a vibrant yellow hue was noted, a phenomenon also documented by Dada *et al.* (2018). Similarly, when leaf extracts of *Chromolaena odorata* (CO) were subjected to $AgNO_3$, a noticeable shift in colour from light-green to a rich brown shade was observed, mirroring the findings reported by Akinnuoye *et al.* (2020). Furthermore, the application of silver nitrate to *Solenostemon monostachyus* (SM) resulted in a change from colourless to a deep, dark-brown colouration. This alteration in colour was also highlighted in the research conducted by Karu *et al.* (2020), where a transition from a pale-yellow hue to a reddish-brown shade was documented. These colour changes serve as key indicators of the successful formation of silver nanoparticles in the respective plant extracts, providing valuable insights into the synthesis process.

Tithonia diversifolia *Solenostemon monostachyus* *Chromolaena odorata*

Plate 1: Formation of silver nanoparticles was identified by colour change (a) leaf extract (b) after the reduction of silver nitrate

Transmission Electron Microscopy of synthesised AgNPs

The morphological and size characteristics of the synthesised silver nanoparticles were examined and analysed using Transmission Electron Microscopy (TEM). The TEM image revealed the presence of dominant spherical nanoparticles in the sample labeled as TD, with an average size of 49.64 nanometres. In contrast, the sample labeled as CO exhibited a mixture of dominant spherical nanoparticles and triangular and rod-shaped nanoparticles, with an average size of 27.48 nanometres. The sample labeled as SM contained predominantly spherical nanoparticles, with an average size of 25.76 nanometres, as shown in Plate 2. This detailed characterisation of the silver nanoparticles provides valuable insight into their structural properties and can aid in further research and applications in the field of nanotechnology.

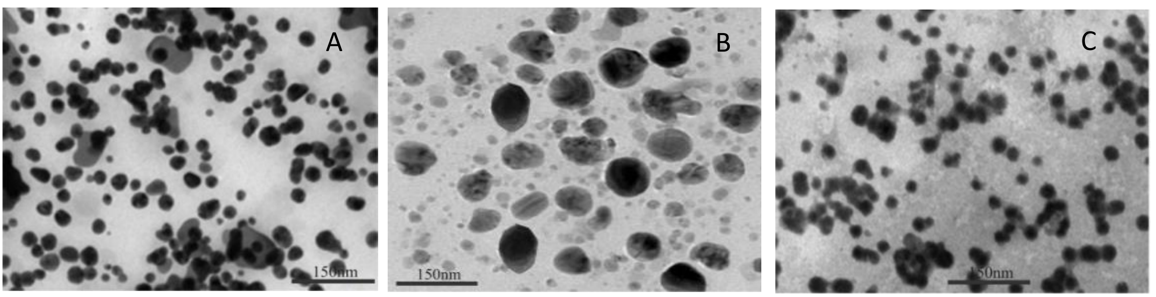

Plate 2: TEM image of AgNps synthesised using leaf extracts of (a) *Chromonaela odorata* (b) T*ithonia diversifolia* (c) *Solenostemon monostachyus*

Fourier Transform Infrared (FTIR) Spectra of synthesised AgNPs

The Fourier Transform Infrared (FTIR) spectra of the bio-reduced silver nanoparticles, which were recorded in the range of 500 cm^{-1} to 4000 cm^{-1}, are shown in Figure 1. This analytical technique was employed to effectively identify the various functional groups present in the silver nanoparticles that were synthesised using leaf extracts. Specifically, the FTIR spectrum of the silver nitrate ($AgNO_3$) precursor exhibited distinct peaks at 1641.16 cm^{-1}, 2062.27 cm^{-1} and a broad peak ranging from 3230.61 cm^{-1} to 3629.55 cm^{-1}. These peaks correspond to the carbonyl stretch of amides, the triple bonded carbon and the stretching vibration of the O–H functional group, as reported by Nandiyanto *et al*. (2019). These functional groups play a crucial role in the bio- reduction process of $AgNO_3$ to silver nanoparticles (AgNPs), as well as in the subsequent capping and stabilisation synthesized using the pod extract of *Cola nitida*. It is worth noting that the leaf extract of TD has been previously documented to contain a variety of bioactive compounds such as saponins, phenolics, flavonoids, alkaloids and tannins, as reported by Omokhua *et al.* (2018). These compounds serve as essential polyols that act as both stabilising and reducing agents in the synthesis and stabilisation of silver nanoparticles. The Fourier-transform infrared (FTIR) spectrum analysis of the synthesised silver nanoparticles (AgNps) using CO as a reducing agent revealed distinct peaks at 1637.99 cm^{-1}, 2062.27 cm^{-1} and a broad peak ranging from 3230.61 cm^{-1} to 3601.06 cm^{-1}. These peaks correspond to the carbonyl stretch of amides, the triple-bonded carbon, and the stretching vibration of the O–H functional group, as reported by Nandiyanto *et al*. (2019). This observation suggests that proteins and phenolic compounds present in the synthesis process acted as capping and stabilisation molecules for the AgNPs. Previous studies by Vijayaraghavan *et al.* (2018) have identified various phytoconstituents such as saponins, phenols, tannins, steroids, flavonoids and terpenoids in the leaf extracts of *Chromolaena odorata*. These phytoconstituents may have contributed to the synthesis and stabilisation of the AgNPs, thereby highlighting the potential role of natural compounds in nanoparticle synthesis and applications.

The Fourier-transform infrared (FTIR) spectrum analysis of the silver nanoparticles (AgNPs) using SM revealed prominent peaks at 1637.99 cm^{-1}, 2059.10 cm^{-1} and 3487.07 cm^{-1}, which could be attributed to the carbonyl stretch of amides, triple-bonded carbon, and stretching vibration of the O–H functional group, as reported by Nandiyanto *et al*. (2019). These peaks indicate the presence of protein-stabilising molecules in the synthesised AgNPs. Previous studies have shown that SM was abundant in various bioactive compounds, including saponins (1.150±0.000 %), tannins (5.443 ±0.065 mg/g), phytate (13.525 ±0.291 mg/g), alkaloids (7.936±2.98 %), oxalate (0.968±0.086 mg/g fresh weight), bioflavonoids (6.16±0.15 mg/g) and total polyphenols (6.03±0.31 mg/g), as reported by Afolabi *et al*. (2021). These findings suggest that SM serves as a rich source of bioactive compounds that may contribute to the synthesis and stabilisation of AgNPs, making it a promising candidate for various biomedical and environmental applications.

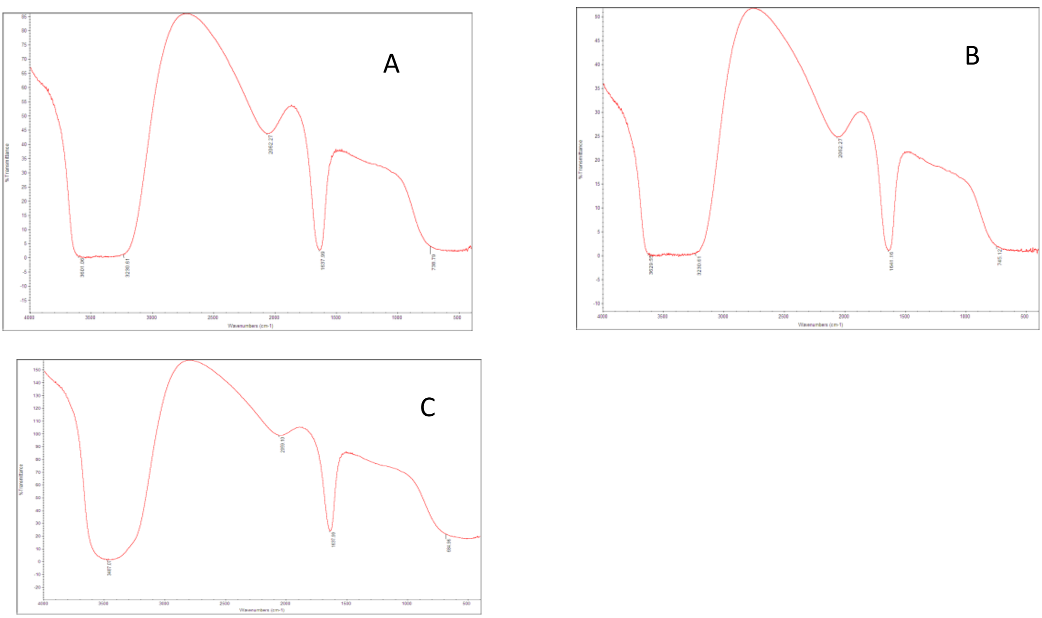

Figure 1: FTIR spectra of AgNPs acquired using leaf extracts of (a) *Chromonaela odorata* (b) T*ithonia diversifolia* (c) *Solenostemon monostachyus* in the range of $500\ cm^{-1} - 4000\ cm^{-1}$

UV–visible Spectroscopy

The confirmation of the formation of silver nanoparticles in an aqueous colloidal solution was achieved through the utilisation of UV-Vis spectral analysis. In Figure 2, the UV-Vis absorption spectra of the synthesised silver nanoparticles solution were examined within the wavelength range of 200 to 1000 nm. It was observed that the absorbance band of the reduced silver sample prominently appeared at 437 nm for TD, 445 nm for CO and 430 nm for SM, all of which were within the wavelength range previously reported for AgNPs by Dada *et al*. (2018). This analysis did not only confirm the successful synthesis of silver nanoparticles but also provided valuable insight into their optical properties and characteristics.

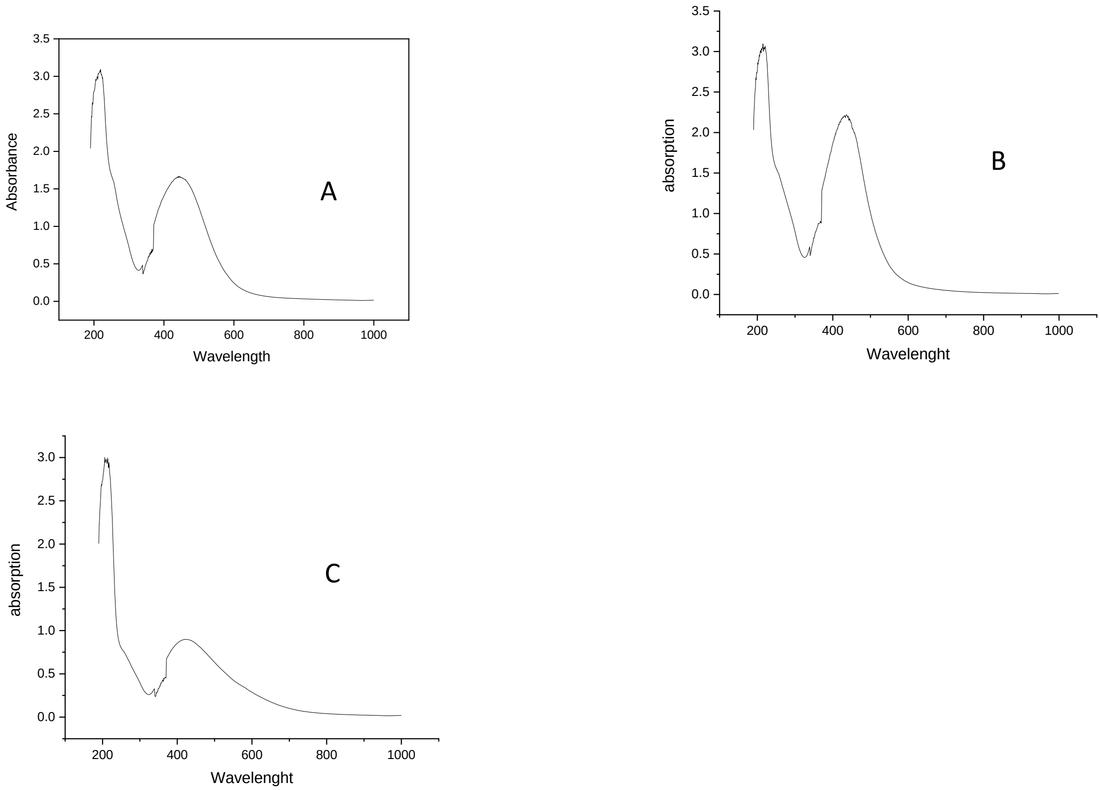

Figure 2: UV-Vis Absorption Spectrum of biosynthesised AgNPs using extracts of (a) *Chromonaela odorata* (b) T*ithonia diversifolia* and (c) *Solenostemon monostachyus*

CONCLUSION

In the realm of nanotechnology, the utilisation of a reliable and environmentally-friendly method for the creation of metallic nanoparticles is an essential prerequisite. Among the various metallic nanoparticles, silver stands out due to its wide array of applications, stemming from its attractive physiochemical properties. In a recent study, conducted with the aim of synthesising silver nanoparticles (AgNPs) without the use of harmful solvents, leaf extracts from *Chromolaena odorata*, *Tithonia diversifolia* and *Solenostemon monostachyus* were employed. The results of the study revealed that the leaf extracts effectively reduced silver nitrate to nano-size, confirming the potentials of natural extracts in the green synthesis of AgNPs.

AUTHORS' CONTRIBUTIONS

Olawepo, G. K. collected plant leaves, processed leaves for extraction and reviewed the manuscript. Adeniran, D. A. and Ekundayo, T. O. conducted the synthesis of silver nanoparticles (AgNPs) and performed comprehensive characterisation using UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM) to analyse their properties. Adewumi, G. A. organised and compiled the research data, as well as the documentation of the findings.

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