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PHYTOCHEMICAL PROFILING AND GREEN SYNTHESIS OF SILVER AND COPPER NANOPARTICLES USING *HIBISCUS SABDARIFFA* AND *XYLOPIA AETHIOPICA*

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Abstract: The increasing demand for eco-friendly nanomaterials has intensified interest in plant-mediated synthesis of metal nanoparticles. This study investigated the phytochemical composition of *Hibiscus sabdariffa* and *Xylopia aethiopica* and their efficiency in the green synthesis of silver (AgNPs) and copper nanoparticles (CuNPs). The objective was to characterize the phytochemicals involved in nanoparticle formation and evaluate the structural and spectral properties of the synthesized nanoparticles. Aqueous extracts of both plants were prepared and subjected to qualitative and quantitative phytochemical screening. Nanoparticles were synthesized by reacting the extracts with $AgNO_3$ and $CuSO_4$ solutions under controlled conditions. UV–Vis spectroscopy, Fourier transforms infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analyses were used for characterization. Results showed high flavonoid (45.80 ± 0.35 mg/g) and phenol (25.50 ± 0.29 mg/g) contents in *H. sabdariffa*, while *X. aethiopica* exhibited higher tannins (25.30 ± 0.27 mg/g) and terpenoids (18.30 ± 0.23 mg/g). Successful nanoparticle formation was indicated by color changes and UV–Vis peaks around 350–450 nm for AgNPs and 350–550 nm for CuNPs. FTIR spectra revealed O–H (3300–3500 cm^{-1}) and C=O (≈1600 cm^{-1}) functional groups responsible for reduction and stabilization. XRD confirmed crystalline structures. Therefore, both plants demonstrated strong reducing and capping abilities, providing a sustainable and non-toxic approach for synthesizing stable Ag and Cu nanoparticles suitable for biomedical and antimicrobial applications.

Keywords: green synthesis, *Hibiscus sabdariffa*, *Xylopia aethiopica*, silver nanoparticles, copper nanoparticles, phytochemicals, spectroscopy.

INTRODUCTION

Nanotechnology has become a cornerstone of modern science, offering innovative solutions across medicine, agriculture, environmental remediation, and materials engineering through the manipulation of matter at the nanoscale (Igiebor *et al*., 2023; Igiebor *et al*., 2024). Among engineered nanomaterials, metallic nanoparticles, particularly silver (AgNPs) and copper nanoparticles (CuNPs), have attracted sustained scientific interest due to their remarkable antimicrobial, antioxidant, catalytic, and antiviral properties (Igiebor & Omoregie, 2024). These attributes have positioned AgNPs and CuNPs as promising candidates for biomedical applications, food preservation, water treatment, and sustainable agriculture

Despite their advantages, conventional physicochemical synthesis methods for metallic nanoparticles are often energy-intensive and rely on hazardous reducing agents, raising concerns about environmental sustainability, human health, and large-scale applicability (Igiebor *et al*., 2024). In response, green synthesis approaches using biological systems have emerged as environmentally benign and cost-effective alternatives. Among these, plant-mediated synthesis is particularly attractive due to its simplicity, scalability, and the inherent richness of plant extracts in phytochemicals capable of acting simultaneously as reducing, stabilizing, and capping agents (Igiebor & Omoregie, 2025; Thatyana *et al*., 2023).

Medicinal plants such as *Hibiscus sabdariffa* L. and *Xylopia aethiopica* (Dunal) A. Rich are widely used in African ethnomedicine and are recognized for their diverse pharmacological properties. *H. sabdariffa* is rich in flavonoids, phenolic acids, anthocyanins, and organic acids, which contribute to its antioxidant, antihypertensive, antimicrobial, and anti-inflammatory activities (Izquierdo-Vega *et al*., 2020; Sapian *et al*., 2023). In contrast, *X. aethiopica* contains significant levels of alkaloids, tannins, terpenoids, saponins, and a characteristic essential oil, supporting its traditional use in the management of infections, inflammation, and gastrointestinal disorders (Yin *et al*., 2019; Macedo *et al*., 2020). The strong redox potential and functional diversity of these phytochemicals make both plants promising biological platforms for nanoparticle synthesis.

Understanding the phytochemical profile of plant extracts is critical for elucidating the mechanisms underlying nanoparticle formation, stability, and functionality. Phenolic compounds and flavonoids are known to facilitate electron donation for the reduction of metal ions, while functional groups such as hydroxyl (–OH), carbonyl (C=O), and aromatic moieties contribute to nanoparticle stabilization and prevent agglomeration (Thatyana *et al*., 2023). Consequently, integrating phytochemical profiling with nanoparticle synthesis provides mechanistic insight into plant–nanoparticle interactions and enhances reproducibility and control of nanoparticle characteristics.

Recent studies have demonstrated the successful green synthesis of AgNPs and CuNPs using various plant extracts, producing nanoparticles with desirable

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physicochemical properties and enhanced bioactivity (Ikhajiagbe *et al*., 2021; Igiebor *et al*., 2024). AgNPs are particularly valued for their broad-spectrum antimicrobial efficacy and wound-healing potential, whereas CuNPs offer economic advantages and exhibit strong catalytic and antimicrobial activities, albeit with higher susceptibility to oxidation (Igiebor & Omoregie, 2025). However, comparative studies that systematically link phytochemical composition to nanoparticle formation efficiency and physicochemical characteristics remain limited, especially for *H. sabdariffa* and *X. aethiopica*.

This study investigated the phytochemical composition of *Hibiscus sabdariffa* and *Xylopia aethiopica* and explores their efficacy in the green synthesis of silver and copper nanoparticles.

MATERIALS AND METHODS

Plant collection and authentication

Hibiscus sabdariffa, and *Xylopia aethiopica* leaves used for the plant-mediated nanoparticles (NPs) were collected from the Botanic garden, University of Benin, Benin City. The plants were identified by a botanist, Prof. H. A. Akinnobosun of the University of Benin, Benin City, Nigeria.

Preparation of plant extracts

Hibiscus sabdariffa leaves were collected and thoroughly washed with distilled water to remove any dust or impurities. The leaves were then being air-dried in a shaded, well-ventilated area to avoid direct sunlight, which can degrade some of the bioactive compounds. The dried leaves were ground into a fine powder using a mechanical grinder. The powders were sieved to obtain uniform particle size. Precisely 50 g of the leaf powder was added to 500 ml of distilled water. The mixture was boiled for 20 minutes and then allowed to cool to room temperature. The extract was filtered using Whatman No. 1 filter paper to remove solid residues.

The dried fruits of *Xylopia aethiopica* were cleaned by gently rinsing with distilled water to remove any surface contaminants. They were allowed to air dry. The dried fruits were ground into a fine powder using a mechanical grinder or mortar and pestle. The powdered fruits were sieved to achieve a uniform particle size. Precisely, 50 grams of the fruit powder was added to 500 ml of distilled water. The mixture was boiled for 20 minutes and then allowed to cool to room temperature. The extract was filtered using Whatman No. 1 filter paper to remove solid residues. The filtered aqueous extract was stored in amber-coloured glass bottles to protect them from light and prevent degradation. The extracts were stored at 4°C in a refrigerator until further use in nanoparticle synthesis.

Phytochemical Screening (Qualitative and Quantitative)

Phytochemical screening was conducted to determine the presence and relative abundance of major bioactive constituents in the aqueous extracts of *Hibiscus sabdariffa* and *Xylopia aethiopica*. Both qualitative and quantitative assessments were carried out using standard analytical protocols as described by Harborne (1973), Trease and Evans (1989), and Sofowora (1993). All chemicals and reagents used were of analytical grade and procured from Sigma-Aldrich. Results were measured in mg/g dry weight (mg/g DW).

The qualitative screening involved the use of specific colorimetric and precipitation reactions to identify major phytochemical groups. Tests performed included Dragendorff's and Mayer's tests for alkaloids, ferric chloride and lead acetate tests for phenols and tannins, frothing tests for saponins, Shinoda and alkaline reagent tests for flavonoids, Liebermann–Burchard reaction for steroids, Salkowski's test for terpenoids, and Keller–Killiani test for cardiac glycosides. Results were recorded as absent (−), present (+), moderately present (++), or highly present (+++) based on the intensity of the reaction observed.

Synthesis protocols for Copper and Silver nanoparticles

For copper nanoparticles (CuNPs), a 50 mL of *Hibiscus sabdariffa* extract with 50 mL of 5mM copper sulphate solution was dispensed into a beaker. The mixture was stirred continuously using a magnetic stirrer. The colour change from blue to greenish-brown, indicate the formation of CuNPs. The mixture was heat on a hot plate at 70°C for 2 hours to ensure complete reduction of copper ions. After the reaction, the solution was cooled to room temperature. To purify the nanoparticles, the reaction was mixed at 10,000 RPM for 20 minutes to separate the nanoparticles from the solution. The supernatant was discarded and he pellet was washed with distilled water to remove any impurities. The washing process was repeated three times. The purified nanoparticles was dried in an oven at 60°C overnight (Igiebor *et al*., 2024).

Silver nanoparticles (AgNPs) were synthesized at room temperature from $AgNO_3$ solution using the extract. Exactly, 5 ml (5 mM) aqueous solution of AgNO3 was added to 20 ml of 5 mg/ml of the extract and stirred for 5 min. The solution turned yellowish brown upon stirring. Then, 0.8 ml of 1M NaOH was added to the solution and stirred for another 5 min. The solution turned to dark brown colour. The colour change is an indication of the formation of silver nanoparticles. Continue stirring the mixture for 1-2 hours to ensure complete reaction (Balavandy *et al*., 2014). The NPs will be washed and purified using centrifuge to separate the silver nanoparticles from the mixture. The NPs will be washed with deionized water to remove any residual reactants. The silver nanoparticles were dried at a specified temperature (Iravani *et al*., 2014; Abbas *et al*., 2024).

Characterization of Nanoparticles

Ultraviolet–visible (UV–Vis) spectrophotometric analysis was used to confirm nanoparticle formation by monitoring surface plasmon resonance (SPR), following the method of Igiebor et al. (2024) with minor modifications. Synthesized nanoparticle suspensions were appropriately diluted with distilled water, and absorbance spectra were recorded in quartz cuvettes over a wavelength range of 300–800 nm at room temperature using distilled water as the blank.

The formation of silver nanoparticles (AgNPs) was confirmed by characteristic SPR peaks between 350–450 nm, while copper nanoparticles (CuNPs) exhibited broader absorption bands within 300–600 nm. Peak position and width were used to infer nanoparticle size distribution and aggregation behavior.

Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify functional groups involved in nanoparticle reduction and stabilization. Dried nanoparticle samples were prepared using the KBr pellet method and analyzed in the spectral range of 4000–400 cm^{-1}. Major absorption bands corresponding to hydroxyl (–OH), carbonyl (C=O), and C–O stretching vibrations were observed, indicating the involvement of plant-derived phytochemicals such as phenols, flavonoids, and terpenoids in nanoparticle capping. Shifts in peak positions relative to crude extracts confirmed interactions between biomolecules and nanoparticle surfaces.

X-ray diffraction (XRD) analysis was carried out to determine the crystalline nature and phase composition of the synthesized nanoparticles using Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 30 mA. Diffractograms were recorded over a 2θ range of 10–80° with a scan rate of 0.02°. The average crystallite size was estimated using the Debye–Scherrer equation. The presence of sharp diffraction peaks confirmed the crystalline structure of the nanoparticles, while minor additional peaks were attributed to residual phytochemical components from the plant extracts. Crystallite size was calculated using the Debye–Scherrer equation:

$$D = \frac{0.9\lambda}{\beta\cos\theta}$$

where D is the crystallite size (nm), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) of the most intense diffraction peak (in radians), and θ is the Bragg angle. .

RESULTS AND DISCUSSION

The qualitative phytochemical analysis (Table 1) of *Hibiscus sabdariffa* and *Xylopia aethiopica* revealed significant variation in the presence and concentration of bioactive compounds. Alkaloids were moderately present in *Hibiscus sabdariffa* but only present in low amounts in *Xylopia aethiopica*. Flavonoids were highly present in *Hibiscus sabdariffa* and moderately present in *Xylopia aethiopica*. Both plants contain tannins, with *Xylopia aethiopica* showing a higher concentration compared to the moderate presence in *Hibiscus sabdariffa*. Saponins are present at low levels in both plants. Phenols were highly present in *Hibiscus sabdariffa* and moderately present in *Xylopia aethiopica*. Glycosides were absent in *Hibiscus sabdariffa* but present in *Xylopia aethiopica*. Steroids and terpenoids show low to moderate presence in both plants, with terpenoids being absent in *Hibiscus sabdariffa* but highly present in *Xylopia aethiopica*.

Table 2 compared the quantitative parameters of various phytochemicals present in *Hibiscus sabdariffa* and *Xylopia aethiopica*. The alkaloid content was notably higher in *Hibiscus sabdariffa* (10.25±0.11 mg/g DW) compared to *Xylopia aethiopica* (5.32±0.08 mg/g DW). Similarly, *Hibiscus sabdariffa* contains a higher concentration of flavonoids (45.80±0.35 mg/g DW) than *Xylopia aethiopica* (20.67±0.21 mg/g DW). However, tannin levels were more prominent in *Xylopia aethiopica* (25.30±0.27 mg/g DW) compared to *Hibiscus sabdariffa* (15.90±0.24 mg/g DW). In terms of saponins, *Xylopia aethiopica* (9.56±0.10 mg/g DW) had a slightly higher concentration than *Hibiscus sabdariffa* (8.75±0.09 mg/g DW). Phenolic content was greater in *Hibiscus sabdariffa* (25.50±0.29 mg/g DW) as compared to *Xylopia aethiopica* (14.85±0.19 mg/g DW). Glycosides were absent in *Hibiscus sabdariffa* but present in *Xylopia aethiopica* (7.50±0.15 mg/g DW). Steroid content was higher in *Hibiscus sabdariffa* (7.20±0.12 mg/g DW) compared to *Xylopia aethiopica* (5.80±0.09 mg/g DW). Terpenoids were absent in *Hibiscus sabdariffa* but significantly present in *Xylopia aethiopica* (18.30±0.23 mg/g DW).

Table 1.

Qualitative parameters of *Hisbiscus sabdariffa* and *Xylopia aethiopica*

Phytochemicals	*Hisbiscus sabdariffa*	*Xylopia aethiopica*
Alkaloids	++	+
Flavonoids	+++	++
Tannins	++	+++
Saponins	+	+
Phenols	+++	++
Glycosides	-	+
Steroids	+	+
Terpenoids	-	+++

Key: - = absent; + = present; ++ = moderately present; +++ = highly present

Table 2.

Quantitative parameters of *Hisbiscus sabdariffa* and *Xylopia aethiopica*

Phytochemicals	*Hisbiscus sabdariffa* (mg/g DW)	*Xylopia aethiopica* (mg/g DW)
Alkaloids	10.25±0.11	5.32±0.08
Flavonoids	45.80±0.35	20.67±0.21
Tannins	15.90±0.24	25.30±0.27
Saponins	8.75±0.09	9.56±0.10
Phenols	25.50±0.29	14.85±0.19
Glycosides	0.00	7.50±0.15
Steroids	7.20±0.12	5.80±0.09
Terpenoids	0.00	18.30±0.23

Mean±SD

Figure 1 shows the UV-Vis absorbance spectra of synthesized nanoparticles. Red sorrel-silver nanoparticles (AgZNPs) exhibited high absorbance at around 350 nm, gradually decreasing as the wavelength increases up to about 750 nm. Beyond this, a slight increase in absorbance is observed from 850 to 1000 nm, indicating possible nanoparticle aggregation or specific surface plasmon resonance behavior of the silver nanoparticles at these wavelengths. Red sorrel-copper nanoparticles (CuZNPs) showed a high absorbance starting near 350 nm and maintain relatively consistent absorbance until around 550 nm, after which the absorbance starts decreasing sharply, indicating the characteristics of the copper nanoparticles. Udder-silver nanoparticles (AgXNPs) exhibited an initial high absorbance at shorter wavelengths (around 350 nm) similar to AgZNPs but experience a much sharper decline around 450 nm. This suggests reduced stability or size changes in the AgXNPs nanoparticles. Udder-copper nanoparticles (CuXNPs) started with relatively lower absorbance compared to CuZNPs at 350 nm and exhibited a sharp decrease, reaching negative absorbance values around 500 nm. This indicates significant structural or chemical changes in the copper nanoparticles, potentially due to the synthesis or environmental conditions.

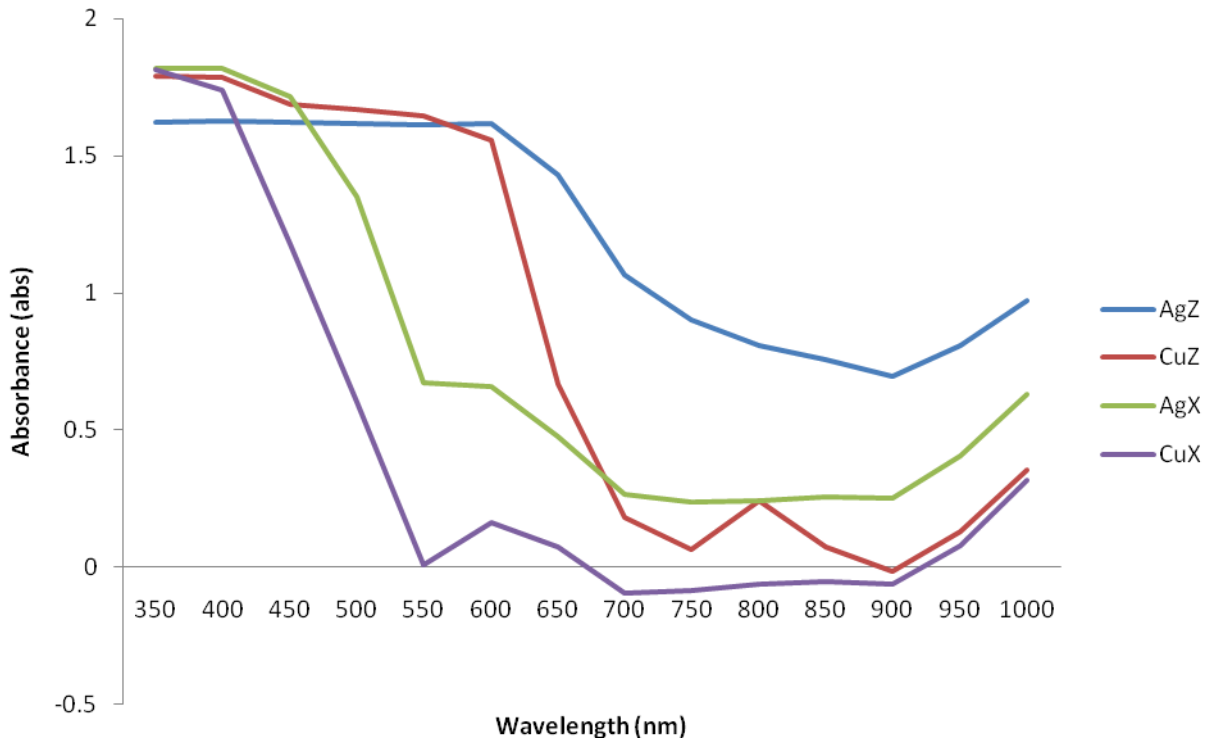

Fig. 1. UV-characterization of synthesized nanoparticles.

Figure 2 shows the FTIR spectra of silver-red sorrel nanoparticles (AgZ) revealed distinct peaks corresponding to functional groups likely involved in the reduction and stabilization of the nanoparticles. Broad absorption bands typically between 3300–3500 cm^{-1} may indicate O-H stretching vibrations, suggesting the presence of phenolic or alcohol groups. Peaks around 1600 cm^{-1} correspond to C=O stretching, which could indicate the presence of carbonyl groups from plant compounds responsible for capping the nanoparticles. These functional groups highlight the role of phytochemicals in nanoparticle formation.

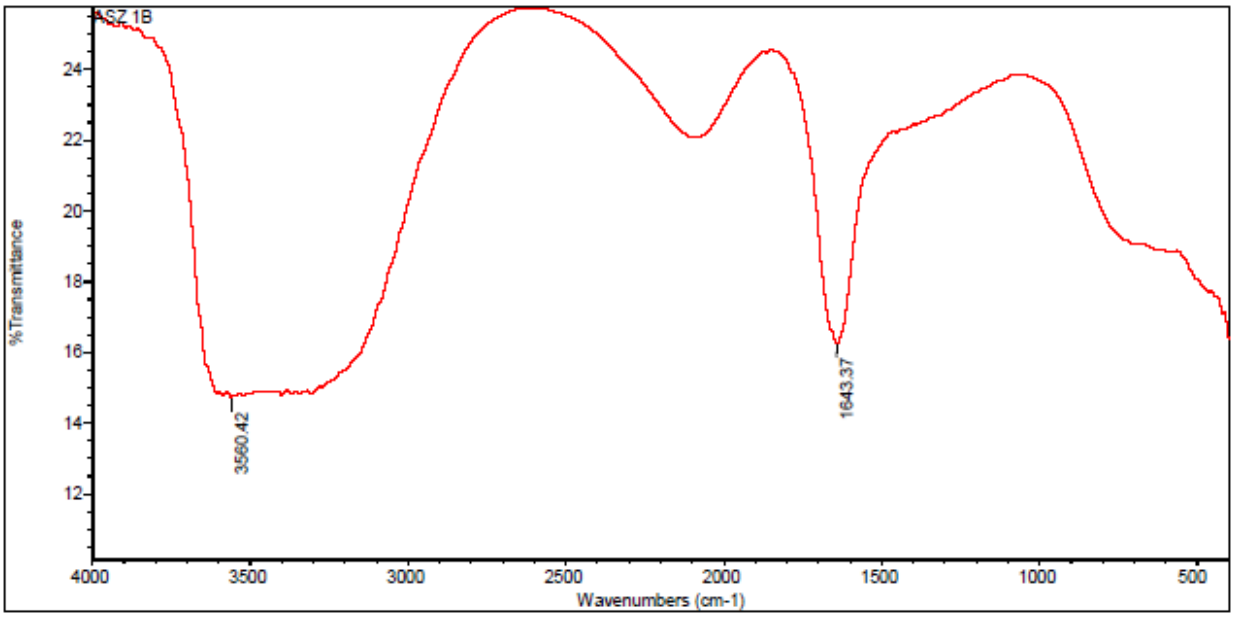

Fig. 2. FTIR spectra of Silver-red sorrel nanoparticles (AgZ).

Figure 3 shows the FTIR spectra of silver-udder nanoparticles (AgX) similarly show characteristic peaks, particularly in the O-H and C=O stretching regions, suggesting that phytochemicals from Xylopia aethiopica also contribute to nanoparticle synthesis and stabilization. Peaks in the region of 1400–1600 cm^{-1} could be attributed to aromatic rings or C=C stretching, indicating the involvement of organic compounds in the silver nanoparticle formation process.

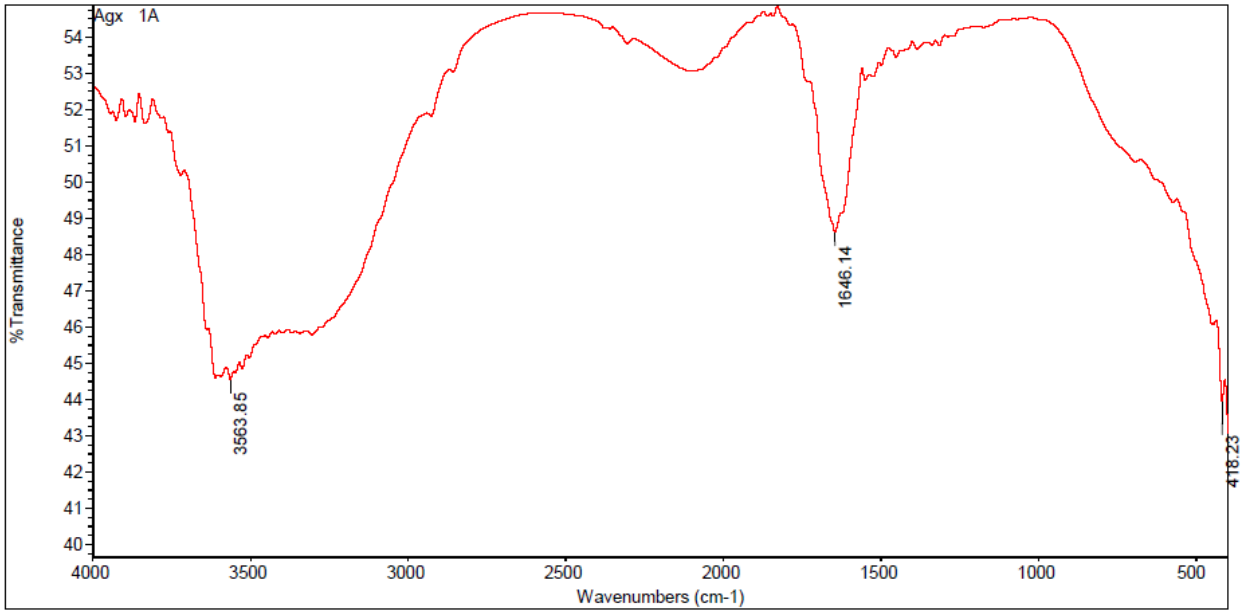

Fig. 3. FTIR spectra of Silver-udder nanoparticles AgX.

In the case of copper-red sorrel nanoparticles (CuZ), the FTIR spectra (figure 4) exhibit peaks in similar regions as those of silver nanoparticles, with notable bands around 3300 cm^{-1} (O-H stretch) and 1600 cm^{-1} (C=O stretch). These findings suggest that red sorrel is equally effective in reducing copper ions and stabilizing the nanoparticles, with phenolic groups and carbonyl compounds playing critical roles in this process.

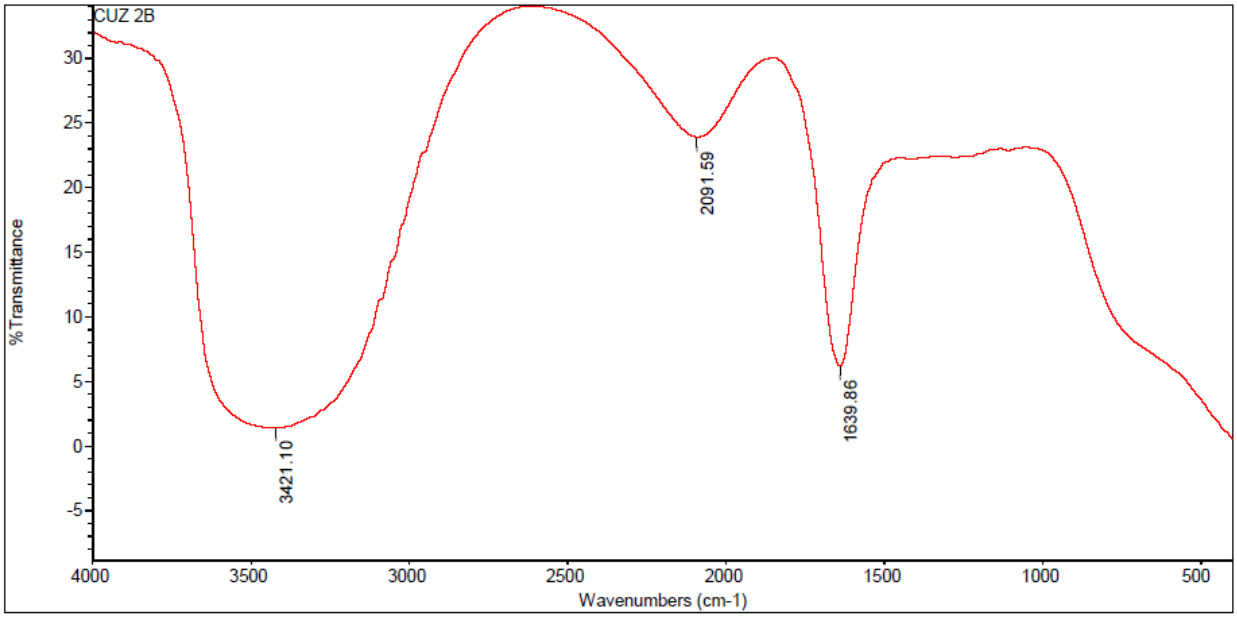

Fig. 4. FTIR spectra of Copper-red sorrel nanoparticles CuZ.

The FTIR spectra (figure 5) of copper-udder nanoparticles (CuX) demonstrate absorption bands around the same regions as the other nanoparticle types. The presence of strong peaks around 3300 cm^{-1} and 1600 cm^{-1} points to the O-H and C=O stretching vibrations, indicating that Xylopia aethiopica contributes functional groups for reducing copper ions and forming stable nanoparticles. Additional peaks between 1000–1500 cm^{-1} may suggest the presence of C-O stretching, which could indicate the involvement of ester or ether groups in the nanoparticle synthesis process.

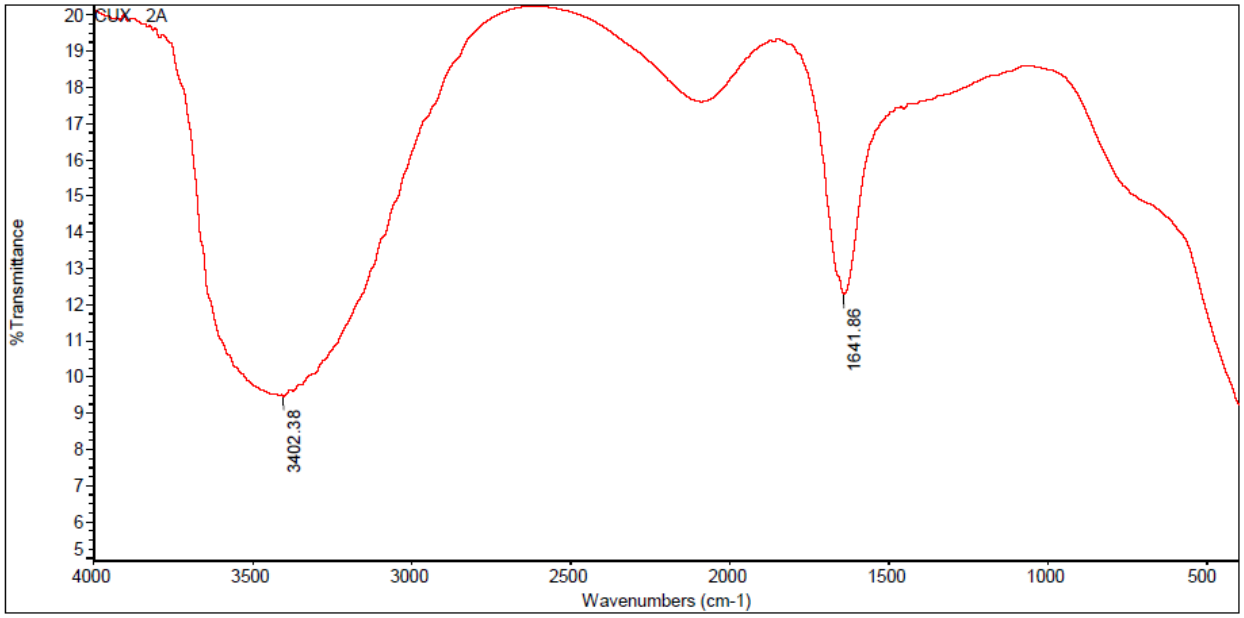

Fig. 5. FTIR spectra of copper-udder nanoparticles CuX.

The analysis of biosynthesized nanoparticles (figure 6) reveals the presence of multiple crystalline phases, identified through X-ray diffraction (XRD) patterns. The identified phases include Litharge (PbO), Challacolloite ($K_2Pb_2Cl_5$), and Quartz (SiO_2), each corresponding to distinct diffraction peaks. The most intense peak is observed for Litharge at 37.969° 2θ, with a notable intensity of 644 cps, indicating its significant presence in the nanoparticles. Challacolloite, a lead-chlorine compound, was also detected, contributing to two significant peaks at 27.96° and 37.969° 2θ. The phase distribution suggests that the nanoparticles were predominantly composed of these lead-based compounds, with quartz appearing as a secondary component.

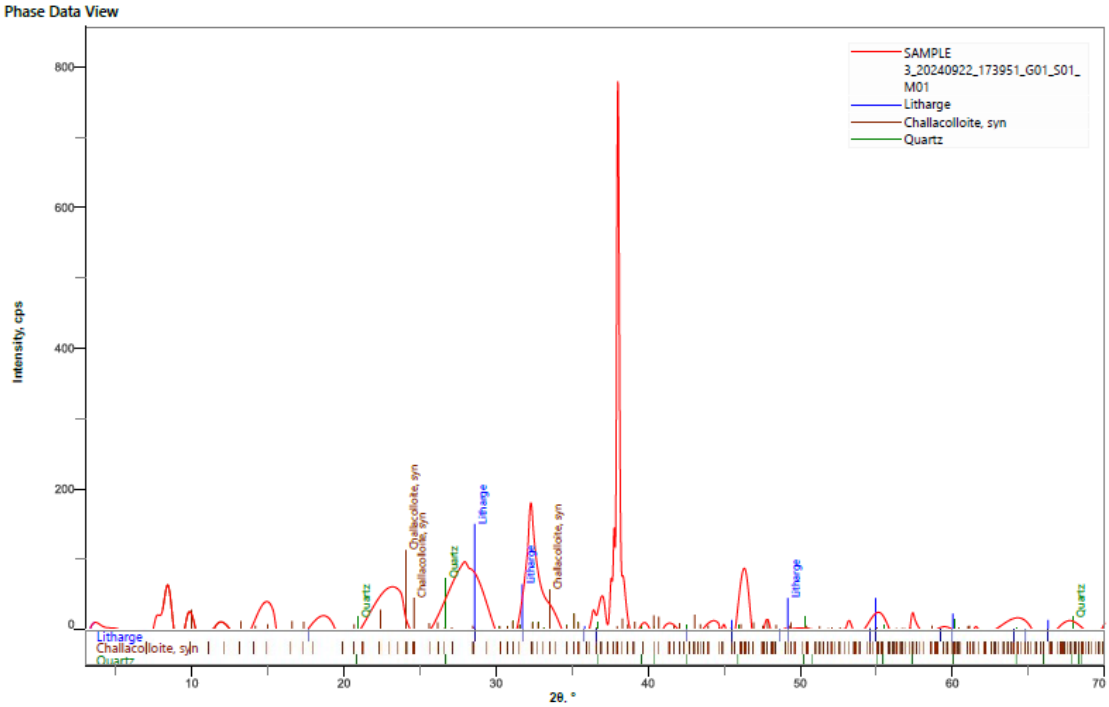

Fig. 6. XRD qualitative analysis of synthesized nanoparticles.

DISCUSSION

This study demonstrated that the phytochemical composition of *Hibiscus sabdariffa* (red sorrel) and *Xylopia aethiopica* (udder) significantly influenced both pharmacological potential and green nanoparticle synthesis. *H. sabdariffa* contained moderate alkaloid levels, suggesting stronger antimicrobial and analgesic activity compared to the low alkaloid content in *X. aethiopica*, aligning with reports of its antihypertensive and diuretic effects by Ghaly *et al*., (2024) and Igiebor & Omoregie (2024). Flavonoids were abundant in *H. sabdariffa*, reinforcing potent antioxidant and anti-inflammatory properties, whereas *X. aethiopica* exhibited moderate flavonoids, consistent with traditional use in inflammation and infection management but suggesting comparatively lower antioxidant potential (Sapian *et al*., 2023; Macedo *et al*., 2020). Tannins were higher in *X. aethiopica*, supporting astringent and antimicrobial applications, while moderate tannin levels in *H. sabdariffa* underpin gastrointestinal benefits (Osei-Asare *et al*., 2025; Guimarães *et al*., 2023). Glycosides were present in *X. aethiopica* but absent in *H. sabdariffa*, reflecting the former's cardioprotective and anti-inflammatory activity. Steroids and terpenoids were detected at low-to-moderate levels in both plants, with terpenoids notably higher in *X. aethiopica*, supporting potent antimicrobial properties (Fleischer *et al*., 2008; Nunes *et al*., 2020).

UV–Vis spectroscopy confirmed successful nanoparticle synthesis. Ag nanoparticles from *H. sabdariffa* (AgZNPs) showed stable SPR peaks around 350 nm with minimal aggregation, whereas AgXNPs displayed sharper declines at 450 nm, indicating lower stability due to size variation or extract composition. Cu nanoparticles from *H. sabdariffa* (CuZNPs) exhibited broad SPR bands up to 550 nm, reflecting stable particle formation, whereas CuXNPs showed negative absorbance around 500 nm, suggesting oxidation or structural degradation (Badran & Hamed, 2024; Igiebor & Omoregie, 2025).

Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed consistent O–H (3300–3500 cm^{-1}) and C=O (~1600 cm^{-1}) stretching across all nanoparticles, highlighting the essential role of phenolic and carbonyl groups in reducing metal ions and stabilizing nanoparticles (Iravani *et al*., 2014; Millavithanachchi *et al*., 2025). Additional C–O stretching (1000–1500 cm^{-1}) in CuXNPs reflects *X. aethiopica*-derived esters or ethers contributing to copper nanoparticle formation (Zhang et al., 2020; Abuzeid et al., 2023).

X-ray Diffraction (XRD) patterns indicated multiple crystalline phases, predominantly litharge (PbO), with challacolloite ($K_2Pb_2Cl_5$) and quartz (SiO_2), demonstrating the biosynthetic method promotes halide incorporation and crystallization, while plant-derived silica contributes secondary phases (Takahashi *et al*., 2023; Abuzeid *et al*., 2023).

Collectively, these results highlight that *H. sabdariffa*, rich in flavonoids and phenolics, produces more stable Ag and Cu nanoparticles, while X. aethiopica contributes unique bioactives such as glycosides and terpenoids, enhancing antimicrobial and cardioprotective potential. The integration of plant metabolites in nanoparticle synthesis not only stabilizes particles but also confers biofunctional properties, emphasizing the strategic selection of plant sources to optimize both nanoparticle stability and therapeutic potential for biomedical and environmental applications.

CONCLUSIONS

This study demonstrated that *Hibiscus sabdariffa* and *Xylopia aethiopica* possess rich phytochemicals capable of reducing and stabilizing silver and copper ions for efficient green nanoparticle synthesis. Formation of AgNPs and CuNPs was confirmed by

characteristic color changes and UV–Vis spectral peaks, while FTIR analysis indicated that functional groups such as hydroxyl and carbonyl played key roles in capping the nanoparticles. The crystallinity observed through XRD further validated successful synthesis. Therefore, the results showed that both plants are effective, sustainable, and low-cost sources for producing biogenic nanoparticles with potential antimicrobial and biomedical applications. Further studies on biological activity and toxicity are recommended to enhance their practical applicability.

AUTHORS CONTRIBUTIONS

Conceptualization: F.A.I. and P.N.S.; methodology, F.A.I.; data collection P.N.S.; data validation, F.A.I., and P.N.S.; data processing F.A.I.; writing—original draft preparation, F.A.I. and P.N.S.; writing—review and editing, F.A.I.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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