# Green Synthesis and Characterization of Silver-Copper Bimetallic Nanoparticles Using *Alternanthera brasiliana* Stem Extract


## ABSTRACT

The green synthesis of bimetallic silver copper nanoparticles (Ag-CuNPs) has gained attention due to their broad applications. Here, Ag-Cu bimetallic nanoparticles were synthesized using an aqueous extract from the stem of *Alternanthera brasiliana*. The phytochemical composition of the extract was evaluated through standard phytochemical tests, and the formation of nanoparticles was tracked using UV-visible spectroscopy. The resulting nanoparticles were further characterized using Fourier Transform Infrared (FTIR) spectroscopy, and X-ray diffraction (XRD). Using the Debye-Scherrer formula to interpret XRD data, the estimated sizes of the silver, copper, and silver-copper nanoparticles were 11.81 nm, 55.33 nm, and 12.45 nm, respectively, all synthesized under comparable conditions. Additionally, the antimicrobial properties of these nanoparticles were evaluated, revealing significant activity against various selected microorganisms. The study highlights an eco-friendly approach for synthesizing Ag-CuNPs with promising antimicrobial potential.



*Keywords: Alternanthera brasiliana, green synthesis, Ag-CuNPs, XRD, antimicrobial activity*

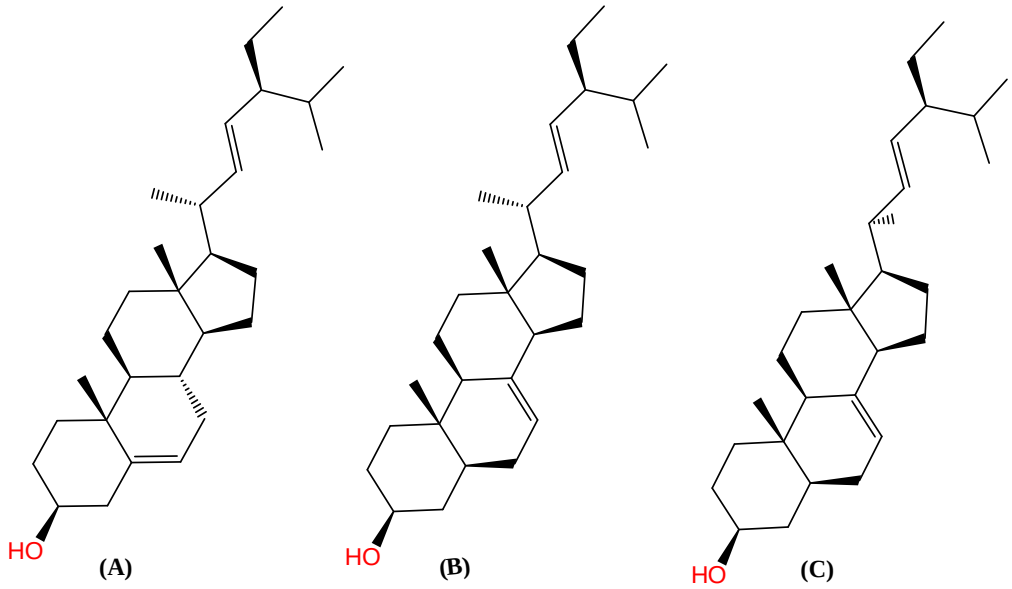

## 1. INTRODUCTION

Nanoparticles have idiosyncratic properties due to profound surface-to-volume ratio and peculiar quantum confinement [1, 2]. These captivating activities have led to their applications in various fields such as medicine, pharmaceuticals, drug delivery, antifungal and antibacterial, etc. [3]. The synthesis of metal and metal oxide nanoparticles for targeted applications is in focus. Synthesis of silver and copper nanoparticles has raised attention over the last decade because of the low cost and great applications in optical, heat systems, catalysts, and sensor properties, and also as an antimicrobial agent [4- 6]. Nanoparticles can be synthesized by various methods among which the green technique is most preferred [4, 7]. Preference for the green synthesis method is due to its eco-friendliness, less expensive, and free from toxic contaminants [8]. Herein, the non-toxic, easily available, and abundant phytochemical constituents such as alkaloids, flavonoids, terpenoids, saponins, tannins, etc. are used to reduce the metal ions and stabilize the nanoparticles [9, 10]. The use of phyto-constituents is being greatly popularized nowadays as these offer advantages like antibacterial, natural reducing, and stabilizing capabilities [11, 12].

Silver nanoparticles have been synthesized using *Zingiber officinale* leaves extract [13], *Capsicum annuum* [14], *Azadirachta indica* [15], and *Jatropha curcas* [16] with good antimicrobial activities. Copper nanoparticles synthesized using *Zingiber officinale* rhizome [9], *Citrus medica* [17], *Citrus limon* [18], and *Azadirachta indica* [19] with good antimicrobial activities have been reported. Bimetallic nanoparticles can be synthesized in a non-hazardous, economical, and easy way using phytochemicals [20]. Some researchers have synthesized the Ag-Cu bimetallic nanoparticles using *Phoenix dactylifera* leaves and studied their catalytic and antibacterial activities [21], using *Trigolella foenum* [22], using *Curcuma longa* for the reduction of persistent organic pollutants [23], and *Aerva lanata* extract and evaluate their cytotoxic and antimicrobial activities [24]. Bimetallic nanoparticles have a diverse range of applications across electrical, optical, catalytic, and biomedical fields [20]. It is understood that charge transfer between metal ions can catalyze various chemical reactions. Furthermore, bimetallic nanoparticles exhibit significant potential in nanomedicine and other biomedical applications [25-27]. An example of this is Cu-Fe bimetallic nanoparticles, which have been reported to effectively remediate environmental issues, particularly in removing nitrates from groundwater [28]. Ag-Cu bimetallic nanoparticles synthesized by using *Phoenix dactylifera* leaf extract have shown excellent photocatalytic activity to degrade the methylene blue dye from wastewater [29]. In addition, bimetallic nanoparticles can function as antimicrobial agents in conjunction with bacteria [30]. These nanoparticles disrupt bacterial growth by destabilizing their membranes and generating reactive oxygen species (ROS), which cause DNA damage and interfere with protein function [18,31,32]. Notably, Ag-Cu bimetallic nanoparticles have shown remarkable antimicrobial activity against Gram-positive bacteria [33]. Some pieces of literature have shown the synthesis of Ag-Cu bimetallic nanoparticles. However, Nonetheless, different plants contain different phytochemicals and they have different capacities for the reduction of metal ions and stabilization of nanoparticles [34]. Based on the literature, synthesis of Ag-Cu bimetallic nanoparticles using *Alternanthera brasiliana*

extract has not been conducted. *Alternanthera brasiliana* is a native Nepalese perennial herb planted for ornamental as well as traditional medicinal value points (Figure 1). Stigmasterol, spinasterol, and β-sitosterol as shown in scheme 1 are the reported phytosterol compounds of this plant [35].

**Fig. 1. Vegetative part of the plant**




**Scheme 1: Structural formula of (A) Stigmasterol, (B) Spinasterol, and (C) Beta-sitosterol**

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant material and Preparation of Extract

*Alternanthera brasiliana* stems sample was collected from the Nawalpur District (27°31' N, 83°39'E), Nepal. The sample was washed with tap water followed by distilled water. It was dried for 15 days and converted into fine pieces. Those pieces were crushed into fine powders with the help of an herbal disintegrator. An aqueous extract was prepared by immersing 10 g of powdered material in 150 mL of distilled water for 24 hours. The solution was filtered to obtain the aqueous extract, which was then used as a reducing and stabilizing agent in nanoparticle synthesis. The possible phytochemicals (responsible for reducing and stabilizing nanoparticles) present in aqueous extract were confirmed by respective chemical test methods.

### 2.2 Synthesis of nanoparticles

Silver nanoparticles (AgNPs) were synthesized by stirring a mixture of 20 mL precursor solution (0.1N $AgNO_3$), 60 mL of aqueous extract, and 30 mL of distilled water in a beaker for 40 minutes. The colloid particles produced were centrifuged and collected in a watch glass. The collected mass was washed with ethanol repeatedly to remove the impurities and then

the collected mass was dried. Triplicate experiments for synthesis followed by centrifugation-decantation-washing processes were carried out and the obtained residue was dried and labeled as AgNPs. A similar process was used to synthesize copper nanoparticles (CuNPs) only using 0.1N $Cu(NO_3)_2$ as the precursor solution instead of the silver precursor solution. Likewise, silver-copper bimetallic nanoparticles were synthesized using 20 mL of 0.1N $AgNO_3$ and 20 mL of 0.1N $Cu(NO_3)_2$ mixture as precursor solution, 60 mL of aqueous extract, and 30 mL of distilled water. All synthesis protocols and environments were constant with the AgNPs synthesis process. The dry mass collected was labeled as Ag-CuNPs.

### 2.3 Nanoparticle Characterization

The formation of nanoparticles in the colloidal form in the reaction mixture was monitored using UV–Visible double-beam spectrometer (2802LT, Labtronics). The sample solution in the cuvette was irradiated with electromagnetic radiation of wavelength 200 to 800 nm and the absorbance was recorded. Similarly, the functional groups of AE associated with stabilizing the nanoparticles were identified using Fourier transform infrared (FT-IR) spectroscopy. Solid nanoparticle samples were scanned in the range 4000-400 $cm^{-1}$ with a scan interval of 4 $cm^{-1}$ using a Perkin Elmer 10.6.2 spectrometer at the Department of Chemistry, Amrit Campus, and Kathmandu. The crystallinity of the synthesized nanoparticles was determined using an X-ray diffractometer (Rigaku-D/max 2500 PC diffractometer). Nanoparticles were exposed to incident X-rays (CuK$\alpha$, 1.5406 Å) at a scanning rate of 10°/min from a 10-90° angle and the intensities of diffracted rays at 2θ angle were recorded. The inter-planner spacing, crystal planes, and grain size of the nanoparticles were calculated using Bragg's equation (1) and Debye Sherrer's formula (2), as below [18,36].

$$n\lambda = 2d \sin\theta \quad (1)$$

$$\text{Grain Size } (D) = k\,\lambda/\beta \cos\theta \quad (2)$$

Where, D = grain size, d = inter-planar distance, λ= 1.5406 Å (wavelength of X-ray used), K = constant (equal to 0.94 for cubic crystal system), θ = diffracted angle, β = Full width at half maximum in radian.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical test results

Phytochemicals are the potential reducing and stabilizing agents. To ensure the possible reducing and stabilizing agents, the phytochemical screening of aqueous extract of *Alternanthera brasiliana* stem was performed, and the findings are tabulated in Table 1.

**Table 1. Phytochemicals present in the aqueous extract of *Alternanthera brasiliana* stem**

| S.N. | Phytochemicals | Aqueous Extract |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | - |
| 3 | Carbohydrates | + |
| 4 | Phenol and Tannins | + |
| 5 | Quinones | - |
| 6 | Proteins | - |
| 7 | Saponins | + |
| 8 | Terpenoids | + |
| 9 | Glycosides | - |
| 10 | Fat and Oils | + |

Absence (-), Presence (+)

### 3.2 UV Spectra Analysis

UV absorbance spectra of synthesized nanoparticles obtained due to surface Plasmon resonance with incident UV wave are shown in Figure 2. The maximum absorbance at 425 nm indicated the formation of AgNPs. Similar findings were reported by Tamilarasi and co-workers in which the maximum absorption peak was observed around (425 nm) [37]. Similarly, the maximum absorbance at 487 nm indicated the formation of CuNPs. Similar findings were reported by Sowbaraniya and co-workers in which the maximum absorption peak was observed at 480-515 nm [38]. Likewise, the maximum absorbance at 412 nm indicates the formation of Ag-Cu NPs. The UV spectral lines due to the surface Plasmon

resonance of the bimetallic nanoparticle are broad in comparison to individual nanoparticles. This implies that the bimetallic nanoparticle showed an effect for broad spectrum indicating the broad spectrum interaction with the light.

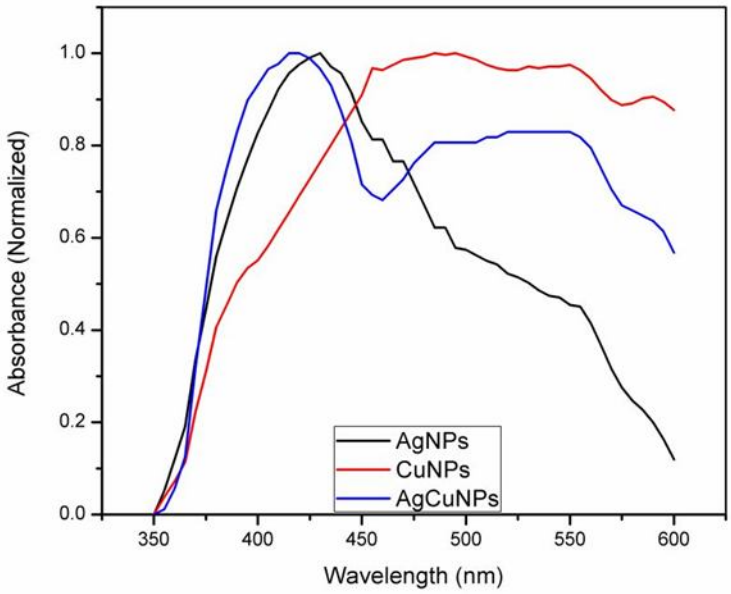



**Fig. 2. Combined UV spectra of AgNPs, CuNPs, and AgCuNPs**

### 3.3 FT-IR Spectra Analysis

FTIR spectroscopy is a powerful tool for the identification of functional groups. Herein, the functional group associated with capping and stabilizing nanoparticles was identified using this tool. Not all but the majority of the functional groups of AE may be oxidized thereby reducing metal ions to metal nanoparticles. Those oxidized as well as non-oxidized functional groups may be responsible for the stabilization of Ag NPs, Cu NPs, and Ag-Cu NPs nanoparticles. The main functional groups that are associated with stabilizing the AgNPs are O-H, C=C, and C=C conjugated amide groups of C-H showing stretching and bending vibrations at 3475, 3038, 2512, and 796 $cm^{-1}$ respectively [11,36,39]. The main functional groups that are associated with stabilizing the copper nanoparticles are O-H, C-H, and $CH_2$ stretching aliphatic groups, C=C conjugated and alkene of C-H showing stretching and bending vibrations at 3403, 2956, 2708, 2566, and 2356 $cm^{-1}$ respectively [9,39]. Likewise, the main functional groups that are associated to stabilize the silver-copper bimetallic nanoparticles are O-H, C-H, and $CH_2$ stretching aliphatic groups, C=O group of quinone compounds, C=O carbohydrate, aromatic compound, halogen compound (iodo compounds) showing stretching and bending vibrations at 3409, 2888, 1669, 1078, 827 and 626 $cm^{-1}$ [20,39-41]. From Figure 3, it was clear that almost all the functional groups for the reduction and stabilization of these three nanoparticles are the same. It is evident that –OH, -CH, -C=O, C-N, and NH functional groups are the major groups for the stabilization of nanoparticles.

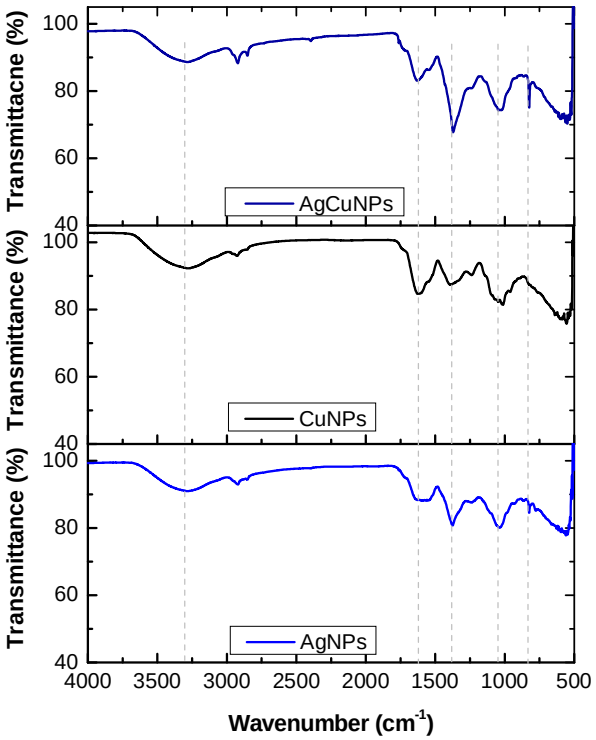



**Fig. 3. Combined FTIR spectra of AgNPs, CuNPs, and Ag-CuNPs**

## 3.4 XRD Spectra Analysis

XRD patterns obtained for AgNPs, CuNPs, and Ag-CuNPs are shown in Figure 4. The diffraction peaks at 2θ value were observed at 27.66, 32.16, 38.15, 46.14, 54.56, 57.28, 65.5, 76.6 and 85.16 for AgNPs (Figure 4(a)). Diffraction peaks at 38.15, 65.5, and 76.6 are (111), (220), (311) crystalline plane of AgNPs (JCPDS No. 04-0783) whereas diffraction peaks at 27.66, 32.16, 46.14, 54.56, 57.28, and 85.16 are (110), (111), (200), (220) and (222) crystalline planes of AgONPs (JCPDS No. 43-0997 and JCPDS No. 43-1038). These oxide peaks may be due to the surface aerial oxidation of nanoparticles. However, Mehta and co-workers reported the crystalline patterns for AgNPs are at 32.35, 46.38, 54.03, and 57.66 [42], and also similar findings are reported by Giri and co-workers for AgNPs [43]. The XRD pattern confirmed that the synthesized AgNPs are a face-centered cubic crystal structure.

The diffraction peaks (2θ values) for CuNPs were observed at 43.06 and 53.36 as shown in Figure 4(b). The diffraction peak at 43.06 and 53.36 have phases of (111) and (220) plane systems (JCPDS No. 04-0836). Similar findings were reported for green synthesized CuNPs in which diffraction peaks were observed at 43.26, 50.32, and 74.05 degrees which indicated the formation of Cu nanoparticles [9, 18, 44]. The sharp peaks of the XRD pattern indicated the crystalline nature of CuNPs. XRD pattern of bimetallic Ag-CuNPs showed diffraction peaks (2θ values) at 28.08, 32.14, 38.15, 43.06, 46.14, 54.6, 57.28, 65.58, 76.6 and 85.16 as shown in figure 4(c) (JCPDS No. 04-0783 and JCPDS No. 04-0836). These 2theta values completely resemble the diffraction angles as shown by AgNPs and CuNPs individually. The diffraction pattern shown by these bimetallic nanoparticles indicates the formation of bimetallic Ag-CuNPs. Rahman and co-workers reported similar findings in which the fcc structure of Ag-CuNPs was validated by five characteristic peaks corresponding to the (111), (200), (220), (222), and (311) planes that occurred at 18, 36, 42, 60, and 64, respectively [45].

The detailed peak position, their miller indices, and the crystal structure for all nanoparticles are shown in Table 2. Similarly, the average grain size determined by applying the most intense peak width (FWHM) in the Debye Scherer's formula is given in Table 3.

**Table 2. d spacing value of synthesized AgNPs calculated using n=1 and λ for CuKα=1.5406Å**

| Nanoparticles | Plane | 2θ (in degree) | θ (in degree) | $d_{hkl}$ = nλ / 2 sin θ (in Å) |
|---|---|---|---|---|
| AgNPs | 111 | 38.15 | 19.075 | 2.36 |
| | 220 | 65.5 | 32.75 | 1.423 |
| | 311 | 76.6 | 38.3 | 1.24 |
| CuNPs | 111 | 43.06 | 21.53 | 2.09 |
| | 220 | 53.36 | 26.68 | 1.71 |
| AgCuNPs | 111 | 38.15 | 19.075 | 2.356 |
| | 111 | 43.06 | 21.53 | 2.098 |
| | 220 | 53.36 | 26.68 | 1.714 |
| | 220 | 65.5 | 32.75 | 1.423 |
| | 311 | 76.6 | 38.3 | 1.242 |
| AgONPs | 110 | 27.66 | 13.83 | 3.22 |
| | 111 | 32.16 | 16.08 | 2.77 |
| | 200 | 46.14 | 23.07 | 1.96 |
| | 200 | 54.56 | 27.28 | 1.68 |
| | 220 | 57.28 | 28.64 | 1.60 |
| | 222 | 85.16 | 42.58 | 1.13 |

**Table 3. The grain size of AgNPs, CuNPs, and AgCuNPs calculated based on the intense peak of XRD spectra**

| Nanoparticles | 2θ (in degree) | β (FWHM, in degree) | β=FWHM (rad) | D = 0.9λ/β cosθ (in nm) |
|---|---|---|---|---|
| AgNPs | 38.15 | 0.732 | 0.01277 | 11.81 |
| CuNPs | 43.06 | 0.156 | 0.00272 | 55.33 |
| AgCuNPs | 38.15 and 43.06 | 0.693 and 0.15 | 0.01209 | 12.45 |

A comparative study of these three nanoparticles based on their XRD pattern gives more detailed information. This indicated that individual graph peaks resemble the combined bimetallic nanoparticle form indicating the formation of bimetallic nanoparticles.

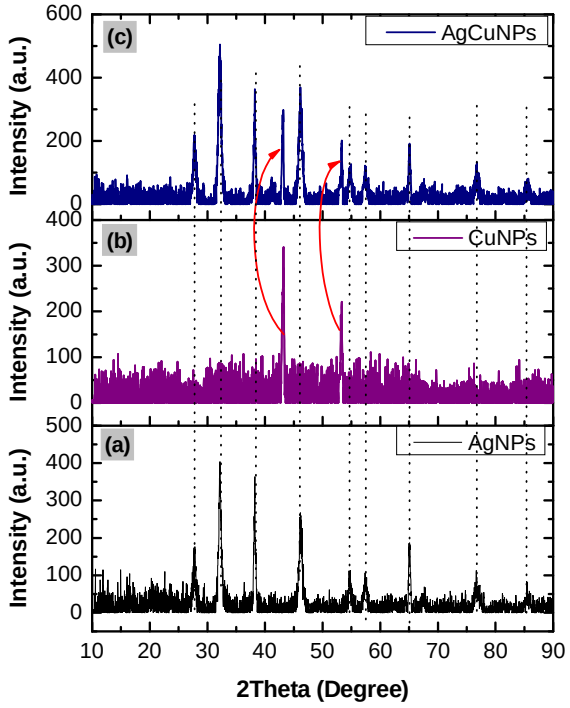



**Fig. 4. XRD pattern of a) AgNPs, b) CuNPs, and c) AgCuNPs comparison**

### 3.5 Antimicrobial Activities of Nanoparticles

The antimicrobial activities of AgNPs, CuNPs, and Ag-CuNPs synthesized using aqueous extract of *Alternanthera brasiliana* stem were carried out against Gram-positive bacteria, Gram-negative bacteria, and fungus (*Candida albicans)* as in Figure 5. The antimicrobial properties of each NPs were measured in terms of the zone of inhibition (ZOI). For this experiment, Kannamycin was taken as standard. According to the results, Ag NPs and Ag-Cu NPs had a reduced antibacterial impact on all microorganisms while Cu NPs had a higher zone of inhibition as in Table 4. The antimicrobial activities of Ag-Cu bimetallic nanoparticles are comparatively lower than individual Ag and Cu nanoparticles. This demonstrated the controlled release of the silver and copper ions in the host cells. This control release mechanism is suitably preferred for long-term protection as well. These findings are recommendable and similar to some other research [21, 24].

**Table 4. Zone of inhibition (ZOI) shown by Ag NPs, Cu NPs, and AgCuNPs**

| Microorganisms (Test organisms) | List of nanoparticles | ZOI of nanoparticles (cm) | ZOI of Standard Kannamycin (cm) |
|---|---|---|---|
| Bacteria<br>Bacillus subtilis (Gram +ve ) | (a) Ag NPs<br>(b) Cu NPs<br>(c) Ag-Cu alloy NPs | 0.6<br>0.7<br>0.5 | 0.9 |
| Escherichia coli<br>(Gram –ve) | (a) Ag NPs<br>(b) Cu NPs<br>(c) Ag-Cu alloy NPs | 0.5<br>1.0<br>0.6 | 1.0 |
| Candida albicans | (a) Ag NPs<br>(b) Cu NPs<br>(c) Ag-Cu alloy NPs | 0.5<br>0.7<br>0.5 | 0.8 |

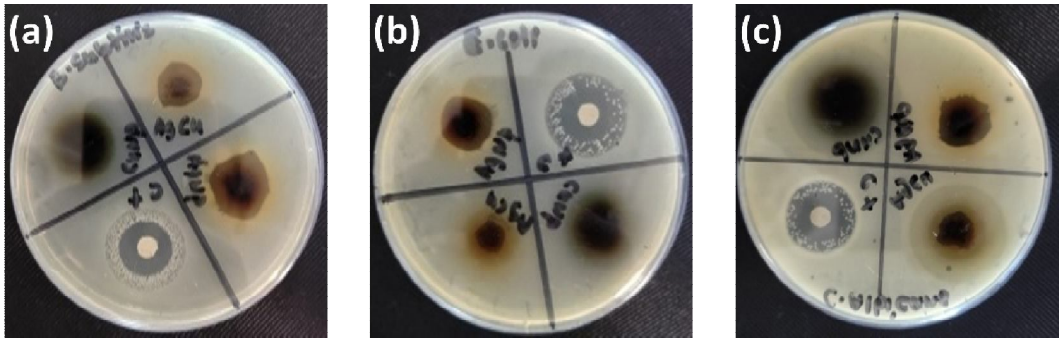



**Fig. 5. Antimicrobial activities of the green synthesized Ag NPs, Cu NPs, and Ag-Cu alloy NPs for a) *Bacillus subtilis*, b) *Escherichia coli*, and c) *Candida albicans***

## *4. CONCLUSIONS*

Aqueous extract of *Alternanthera brasiliana* stem was used in the study to demonstrate a straightforward and environmentally benign process for producing silver, copper, and silver-copper bimetallic nanoparticles. Synthesized nanoparticles were successfully characterized by XRD, FTIR, and UV. UV spectroscopy was employed to observe the nanoparticle formation, whereas FTIR spectroscopy was used to analyze the reactive groups of the phytochemicals responsible for nanoparticle stabilization. The stretching aromatic groups C=C conjugated amide groups of C-H and the -OH, C-O, and $-CH_2$ were detected in the FTIR spectra. The Debye-Scherer formula was used to determine the sizes of silver, copper, and Ag-Cu nanoparticles and was found to be 11.81, 55.33, and 12.45 nm, respectively. Synthesized AgNPs, CuNPs, and Ag-CuNPs were tested for their antimicrobial properties against fungi (*Candida albicans*) Gram-positive *(Bacillus subtilis*), and Gram-negative (*Escherichia coli*) bacteria. This implies that the *Alternanthera brasiliana* stem extract can be suitably used as a capping and reducing agent in the green technique for the synthesis of AgNPs, CuNPs, and Ag-CuNPs.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## AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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