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# Green Synthesis and Characterization of Copper-Zinc Alloy Nanoparticles Using Stem Extract of *Tinospora cordifolia* and Comparative Study of Anti-Microbial Properties

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### Abstract

Bimetallic nanoparticles are prepared from the combination of two different metals in a certain ratio and are getting importance over monometallic nanoparticles due to their better properties. In this work, copper-zinc nanoparticles (Cu-Zn NPs) have been prepared by reducing the metal precursors using *Tinospora cordifolia* (Gurjo) stem extract as a reducing, capping, and stabilizing agent. The physicochemical properties of bimetallic nanoparticles have been studied in terms of UV-vis spectra, XRD and FTIR. Biological activities of as-synthesized nanoparticles were tested against *Bacillus subtilis, Escherichia coli,* and *Candida albicans.* The Cu-Zn NPs exhibited remarkable antibacterial as well as antifungal activities compared to either nanoparticle alone. Based on the findings, it can be revealed that Cu-Zn NPs can serve as good antimicrobial agent.

**Keywords**: *Tinospora cordifolia,* green synthesis, Cu NPs, Zn NPs, Cu-Zn NPs

## 1. Introduction

Bimetallic nanoparticles are getting interest over monometallic nanoparticles in the field of science and technology due to their optimized Plasmon absorption (G. Sharma *et al.*, 2019). A novel approach to vary the properties of particles by blending the metal precursors in different ratio can impart a desired class of properties in bimetallic nanoparticles. Bimetallic nanoparticles with different integration like core-shell NPs, subcluster NPs, multi-shell NPs and alloy NPs have been reported in literatures (Nasrabadi, Abbasi, Davaran, Kouhi, & Akbarzadeh, 2016). Various bimetallic nanoparticles like platinum-, nickel-, iron-, palladium-, gold-based nanoparticles, etc. have been synthesized for their versatile applications. Coinage metal-based nanoparticles are valued for their various applications in various fields like catalysis, nanomedicine, imaging, antibacterial and photo catalysis. Various preparative procedures such as electrochemical reduction, thermal decomposition, hydrothermal method, photochemical decomposition, chemical reduction, sputtering method, sol-gel method, chemical precipitation method, micro-emulsion method, and green synthesis methods have been reported in the synthesis of bimetallic nanoparticles (G. Sharma *et al.*, 2019; Toshima & Yonezawa, 1998). A common and versatile method of bimetallic nanoparticle synthesis includes the reduction of soluble metal salts using suitable reducing agents. In general, bimetallic nanoparticles can be synthesized by simultaneous reduction of two different types of metal ions in presence of suitable reducing agents, capping agents and stabilizing agents. Commonly employed reductants includes glucose, citrate and sodium borohydride (Loza, Heggen, & Epple, 2020). Preparation condition, metal ions concentration, and ambient atmosphere significantly influences the properties of nanoparticles (Bhattarai & Kim, 2020; Maharjan *et al.*, 2020; Rezk, Bhattarai, Park, Park, & Kim, 2020; A. K. Sharma, Rana, Shrestha, Oli, & Bhattarai, 2022). However, rampant uses of chemicals can induce a chemical toxicity and lead to environmental pollution. Therefore, material scientists are searching new and benign methods of nanoparticles synthesis.

One of the environmentally friendly methods of NPs synthesis is green synthesis. In this method, the phytochemicals present in plants are executed for the reduction of metal ions precursors. Such phytochemical constituents are benign, environmentally friendly and of low-cost materials. Zhan *et al.* synthesized Au-Pd bimetallic NPs via single-step bireduction method using *Cacumen platycladi* leaf extract in aqueous environment (Zhan *et al.*, 2011). Similarly, Smuleac *et al.* developed Fe/Pd bimetallic NPs using green tea (*Camellia sinensis*) extracts which consists of a number of polyphenols which can act as reducing, capping and chelating agent (Smuleac, Varma, Sikdar, & Bhattacharyya, 2011). Bimetallic nanoparticles have so many applications such as electrical, optical, catalytic and biomedical. It is believed that charge transfer from one metal ions to another can catalyze many chemical reactions. Besides these, bimetallic NPs have promising uses in nanomedicine and biomedical applications. Cu-Fe bimetallic NPs have been reported for its nitrate clearance in ground water via in-situ remediation (Muradova, Gadjieva, DI PALMA, & Vilardi, 2016). Bimetallic NPs also serve as antimicrobials in combating with bacteria. Such nanoparticles can influence the bacterial growth by unsettling their membrane or by generating reactive oxygen species (ROS) which causes destruction of DNA and impede the protein function. Ag-Cu bimetallic NPs have shown antimicrobial activities against Gram-positive bacteria, *Bacilus subtilis* (Nazeruddin, Prasad, Shaikh, & Shaikh, 2014). In similar work, Ag doped ZnO NPs exhibited antibacterial activity against *Staphylococcus aureus* and *Bascillus subtilis* (N. Sharma, Kumar, Thakur, Sharma, & Shrivastava, 2013). Some literatures show the synthesis of Cu-Zn NPs for their multiple applications. However, the choice of phytochemicals can influence the effectiveness of the NPs. Riaz *et al.* synthesized Cu-Zn NPs using an aqueous extract of *Mirabilis jalapa* and used for dye adsorption as well as antioxidant studies (Riaz *et al.*, 2022). Cu/Zn bimetallic NPs were dispersed over carbon nanofibers and demonstrated its antibiotic activities (Ashfaq, Verma, & Khan, 2016). Though various research works have been performed, there are plenty of space in the synthesis of Cu-Zn NPs using effective phytochemical constituents. To the best of our knowledge, Cu-Zn bimetallic NPs have not been reported using the aqueous extract of *Tinospora cordifolia* (Gurjo) stem. This plant is ubiquitous in the climate of Nepal. Therefore, no special labour cost is required in its growth. The various phytochemicals reported in this plant itself is used for various ailments in Ayurveda. Therefore, this research designs the uses of *Tinospora cordifolia* stem extract for the preparation of Cu-Zn bimetallic NPs. As-prepared NPs exhibited good antibacterial efficacy against Gram-positive and Gram-negative bacteria. Both Cu NPs as well as Zn NPs exhibit antimicrobial activities. It is hypothesized that the Cu-Zn bimetallic NPs would exhibit better antibacterial activities than that by either NPs.


**Fig. 1: *Tinospora cordifolia* (A) Stem (B) Leaves.**

## 2. Materials and methods

### Apparatus and instruments

UV-Vis double beam spectrophotometer (LT-2808), FTIR spectrometer (Perkin Elmer 10.6.2), centrifuge, oven, Grinder, weighing machine, volumetric flasks, pipettes, magnetic stirrer, conical flasks, beakers, glass rod, watch glasses, water bath, etc. were used for this study.

### Chemicals and reagents

Copper(II)sulphate pentahydrate ($CuSO_4.5H_2O$, 99% purity, MW= 249.68 g/mol), extra pure zinc sulphate ($ZnSO_4.7H_2O$, MW= 287.54 g/mol), nutrient agar media for bacteria growth, distilled water, ethanol were received from the laboratory of Amrit Campus and were used as-received without any further refinement. Stem-extract of Gurjo (*Tinospora cordifolia*) was used as reducing and stabilizing agents for the synthesis of copper, zinc, and copper-zinc alloy nanoparticles.

### Test organisms

Antimicrobial activity of the synthesized Cu, Zn, and Cu-Zn nanoparticles were investigated for *Bacillus subtilis* (ATCC 6051) as Gram-positive bacteria, *Escherichia coli* (ATCC

8739) as Gram-negative bacteria, and *Candida albicans* (ATCC 2091 as fungi. *Escherichia coli* is also regarded as Multi-Drug Resistance (MDR) that was used as a test organism (Bhattarai, Shrestha, Shrestha, Park, & Kim, 2018).

### Collection of sample and preparation of extract

*Tinospora cordifolia* (Gurjo) stems were collected from the local field of Kakani Municipality-01. The sample was washed with tap water followed by distilled water. Then, it was dried and chopped into fine pieces. After that, these pieces were ground into fined powder using Herbal Medicine Disintegrator (Model FW177, power 1200W). 150 g of Gurjo stem powder was mixed with 1500 mL distilled water in the large-sized beaker. Then the mixture was heated for about three hours at 60-70 °C in a heating mantle. The hot mixture was left for cooling naturally and filtered off using normal filter paper. Resulting stem extract was stored at room temperature for future use. Phytochemical examinations were carried out for aqueous extracts as per the standard method prescribed by Pharmacologist (Tiwari & Soo Lee, 2013) and chemists (Jain, Nagar, & Devra, 2015). Phytochemical screening of aqueous stem extract was performed as per our previously reported protocols (Oli *et al.*, 2022; Thapa Magar, Budhathoki, Rajaure, Oli, & Bhattarai, 2023; Thapa *et al.*, 2022).

### Green synthesis of copper nanoparticles, zinc nanoparticles and copper-zinc alloy nanoparticles

For the synthesis of copper nanoparticles, 125 mL of stem extract of Gurjo was percolated into 500 mL of 0.1 M $CuSO_4.5H_2O$ aqueous solution and the solution was kept at 80 °C for 3 h with constant magnetic stirring. The suspension was centrifuged at 400 rpm for three hours. The supernatant liquid was decanted off and the residue was washed with distilled water. Centrifugation-decantation-washing processes were performed repeatedly to remove the impurities. As obtained precipitate was dried in an oven at 100 °C for half an hour. Thus, prepared copper nanoparticles were subjected to further characterization and analysis. Zinc nanoparticles was synthesized by similar to the copper nanoparticles synthesis where 0.1 M $ZnSO_4.7H_2O$ aqueous solution was used as zinc precursor. Similarly, copper-zinc nanoparticles was prepared by similar methods to the above method by percolating 125 mL of stem extract of *Tinospora cordifolia* into the mixture of 250 mL 0.1 M $CuSO_4.5H_2O$ and 250 mL 0.1 M $ZnSO_4.7H_2O$ aqueous solutions as salt precursors.

### Physicochemical characterization

The formation of Cu NPs, Zn NPs, and Cu-Zn NPs was assessed by measuring the absorbance of ethanolic dispersion of each kind of NPs taking ethanol as a blank in UV-Visible double beam spectrum over a range of 190-1100 nm at the resolution of 2 nm. For this measurements, dispersed samples of NPs were taken in a quartz cuvette at a sufficient dilution.

For the study of functional group present in the materials, A spectrum GX Fourier transform infrared (FT-IR) spectrometer (Perkin Elmer, USA) was used in the range of 4000–400 $cm^{-1}$. For the study of crystallinity of as-prepared materials, X-ray powder diffraction patterns of each sample were measured using a Rigaku X-ray Diffractometer (Japan) with Cu-Kα (λ = 1.5406 Å) radiation at Bragg's angle (2θ) of 5-90°. Grain size of the synthesized materials was calculated using Debye Scherrer equation, D = k λ /β cos Θ. Here, D is crystallite or grain size (nm), K is Scherrer constant whose value is 0.94, Beta is the full width at half maximum expressed in radian

$\beta = \frac{FWHM \times \pi}{180}$, and λ= 0.15406 nm, CosΘ = Bragg angle.

## Antimicrobial activity

Kirby-Bauer Agar Diffusion method is the method of testing antimicrobial activity, reported in the literature (Hudzicki, 2009; Igbinosa, Ogofure, & Beshiru, 2022). The anti-biotic susceptibility test (AST) measures the ability of antibiotics which means antimicrobial agent inhibits the growth of microbes (bacteria/ fungi). Herein, average diameter of zone of inhibition (ZOI) exhibited by plant extracts on the agar surface against specific pathogenic bacteria is recorded for the estimation of the antimicrobial activity of the plant extract.

**Table 1**: Composition of nutrient agar and nutrient broth

| Ingredients | Nutrient Agar (g/L) | Nutrient Broth (g/L) | Use |
|---|---|---|---|
| Peptic digest of animal tissue | 5.00 | 5.00 | For cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids. |
| Sodium chloride | 5.00 | 5.00 | |
| Beef extract | 1.50 | 1.50 | |
| Yeast extract | 1.50 | 1.50 | |
| Agar | 15.00 | - | |

**Table 2**: Composition of Potato Dextrose Agar and Muller Hinton Agar

| Potato Dextrose Agar | | Muller Hinton Agar | |
|---|---|---|---|
| **Ingredients** | **Gram per liter** | **Ingredients** | **Gram per liter** |
| Potato | 200 g | Beef infusion | 300 g |
| Dextrose | 20 g | Acid hydrolysate of casein | 17.5 g |
| Agar | 15 g | Starch | 1.5 g |
| Distilled water | 1000 mL | Agar | 17.0 g |
| - | - | Distilled water | 1000 mL |

### Collection of test organisms

The active cultures of three microbial strains were employed in the study. The microbial strains were provided by Himalaya Research Institute of Biotechnology Pvt. Ltd. Koteshwor, Kathmandu. The studied strains included two bacteria and one fungus. One of the bacteria was Gram-positive, i.e., *Bacillus subtilis* ATCC 6051 and the next was Gram-negative, i.e., *Escherichia coli* ATCC 8739. The fungal specimen was *Candida albicans* ATCC 2091.

### Screening and evaluation of antimicrobial activity

### Antibacterial test

Previously prepared sterile Muller Hinton Agar plates were dried for the removal of excess moisture from the surface of the media. 95 % alcohol was taken in a beaker and a bent glass rod was dipped into it. Then, 5 µL of standard bacterial culture was kept at the center of labeled plates. A bent glass rod was used to touch the Agar surface slightly. Then the culture was spread over the Agar surface. Later on, the plates were rotated for uniform distribution. These plates were covered with cover and left for few minutes for some minutes.

Filter paper was cut to make a disc of 5 mm diameter and was placed on the surface of media on Petri plates. These plates were labelled well. Later on, 10 mg each of NPs were kept on the respective disc using tweezers. These plates were covered with lids and were kept at room temperature for about half an hour for proper diffusion of the NPs onto the media. These plates were incubated overnight at 37 °C. Then, the ZOI produced by antibacterial activities of plant extract were measured.

### Antifungal Test

Broth culture of fungal strain was inoculated into an already prepared sterile Potato Dextrose Agar (PDA) plate and was spread uniformly. Following this, the disc was placed on the surface of the media and different samples of nanoparticles were loaded on the corresponding disc using tweezers aseptically. Then the plates were covered and put at room temperature for about half an hour for proper diffusion of NPs onto the media. The plates were incubated overnight. Then, the antifungal activity was determined by measuring the zone of inhibition.

## 3. Results and discussion

### Phytochemical screening analysis

The phytochemical screening of stem extract of *Tinospora cordifolia* was performed in aqueous extract qualitatively where a test of proteins, alkaloids, terpenoids, quinones, saponins, polyphenols, glycosides, and flavonoids was carried out. The result of phytochemical screening shows the presence of flavonoids, glycosides, terpenoids, and Quinone. The result of the phytochemical activity of the aqueous extract is shown in table 3.

**Table 3**: Results of Phytochemical Screening of aqueous stem extract

| S.N. | Experiments | Observations | Inferences |
|---|---|---|---|
| 1. | **Test for Flavonoids**<br>5 mL dil. Ammonia solution + extract + conc. sulphuric acid from the side of tube | Yellow ppt was formed. | Presence of flavonoids |
| 2. | **Test for Glycosides**<br>3 drops Molisch`s reagent + 2 mL extract, shaken well + few drops conc. sulphuric acid slowly from the side of tube and allowed to stand for few minutes | Violet ring at junction of two layers was formed. | Presence of glycosides |
| 3. | **Test for Polyphenols**<br>3 drops of 5% $FeCl_3$ + 2 mL extract, shaken well | Black coloration wasn`t formed. | Absence of polyphenols |
| 4. | **Test for Terpenoids**<br>2 mL $CHCl_3$ + 5 mL extract + 3 mL conc. $H_2SO_4$ slowly | Reddish brown coloration was formed. | Presence of terpenoids |
| 5. | **Test or Saponins**<br>5 mL extract + 20 mL distilled water, shaken vigorously | Appearance of frothing was observed. | Presence of saponins |
| 6. | **Test for Quinones**<br>2 mL extract + conc. HCl | Yellow-colored ppt was formed. | Presence of quinones |
| 7. | **Test for Proteins**<br>**Biuret test:** 2 mL 5% NaOH + 2 mL extract + $CuSO_4$ solution | No pink coloration was formed. | Absence of proteins |
| 8. | **Test for Alkaloids**<br>a. Mayer`s test<br>3 drops of Mayer`s reagent + 2 mL extract, shaken well<br>b. Dragendorff`s test<br>3 drops Dragendorff`s reagent + 2 mL extract, shaken well | Yellow ppt was formed.<br>Brown reddish coloration was formed. | Presence of alkaloids<br>Presence of alkaloids |

The results of green synthesis of copper nanoparticles, zinc nanoparticles, and copper-zinc alloy nanoparticles by the reduction of aqueous copper ions and zinc ions using stem extract of Gurjo (*Tinospora cordifolia*) were monitored by color inspection and UV-Visible Spectroscopy.

## Visual observation

After the addition of copper sulphate solution into the Gurjo stem extract, the blue color of copper sulphate pentahydrate got changed into a bluish-green color indicating the formation of copper nanoparticles as depicted in figure 2.

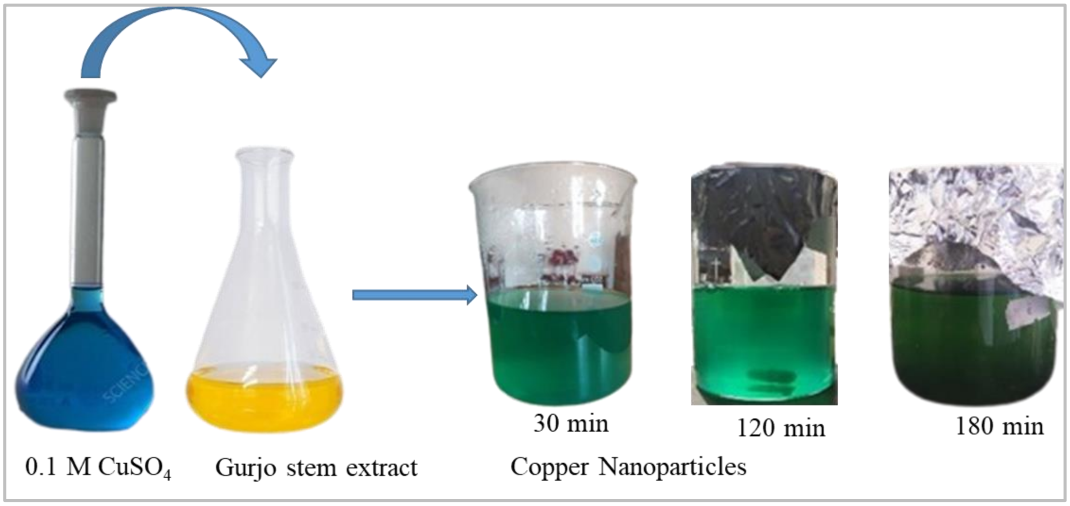


**Fig. 2**: **Digital photographs of copper nanoparticles synthesis by the action of Gurjo stem extract with 0.1 M copper sulphate solution under varied time**.

After the addition of copper sulphate solution into the stem extract of Gurjo, the color of mixture got changed into the light green indicating the beginning of NPs formation. Then the color of the solution gets changed gradually with the passage of time. Finally, a bluish-green color was observed. These changes show that Gurjo stem extract serve as reducing agent in the synthesis of copper nanoparticles. In this work, Gurjo extract completely reduced the 0.1 M copper sulphate within 3 hours to form a copper nanoparticle (Figure 3).

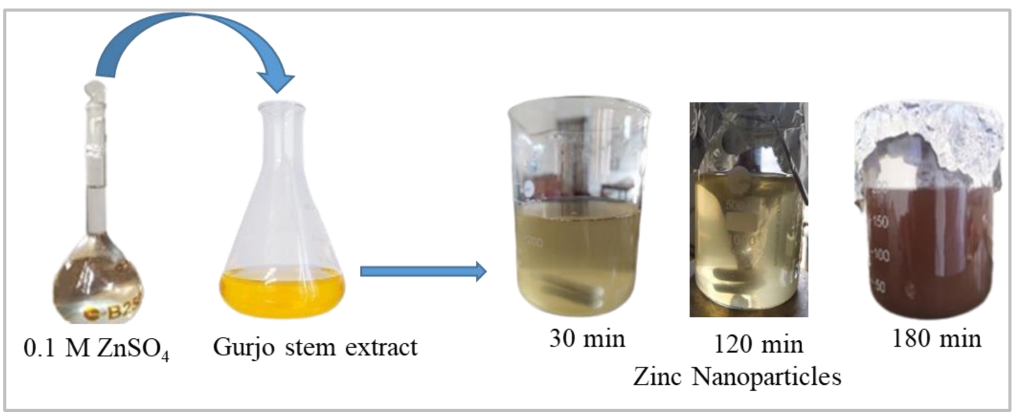


**Fig. 3**: **Digital photographs of copper nanoparticles synthesis by the action of Gurjo stem extract with 0.1 M zinc sulphate solution under varied time.**

In the preparation of zinc nanoparticles, upon addition of zinc sulphate into the orange color Gurjo extract, color of the mixture instantly changes to a pale-yellow color indicating the beginning of zinc sulphate reduction into the zinc nanoparticles. It shows that Gurjo stem extract act as a reducing agent. Then the color of the reaction solution gradually started to change with the increase in time of stirring and finally dirty yellow color solution was obtained. Therefore, Gurjo extract completely reduced the 0.1 M zinc sulphate within 3 hours to form a zinc nanoparticle (Figure 4).


**Fig. 4**: **Digital photographs of copper-zinc nanoparticles synthesis by the action of Gurjo stem extract with 0.1 M zinc sulphate and 0.1 M copper sulphate solution.**

When copper sulphate and zinc sulphate solution are added to the yellowish-orange color Gurjo extract, the color of the mixture immediately appeared to be a pale blue color which indicated that the plant extract began to reduce the zinc sulphate solution. Thus, it is clear that Gurjo stem extract act as a reducing agent. Then the color of the reaction solution gradually started to change with the increase in time of stirring and finally the greenish-blue color solution was obtained. Therefore, Gurjo extract completely reduced the 0.1 M zinc sulphate and 0.1 M copper sulphate within 3 hours to form a zinc nanoparticle (Figure 4).

## UV-Visible absorption spectroscopic studies

UV–Visible absorption spectra of synthesized nanoparticles are demonstrated in Figure 5. These are only preliminary results that are further confirmed by UV-Vis spectroscopic studies. A hump was observed for copper NPs, zinc NPs, and alloy NPs in the range of 365-460 nm.

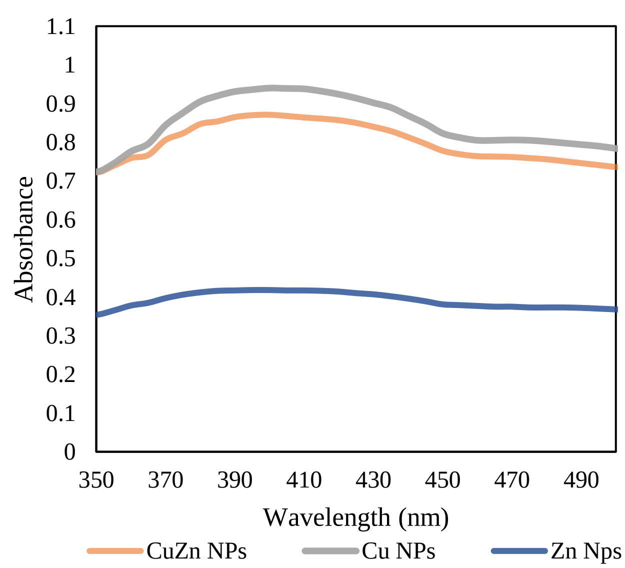


**Fig. 5: Comparative study of UV-Vis of Cu, Zn, and Cu-Zn alloy NPs**

## X-ray diffraction

The crystal structure of as-synthesized NPs was studied in terms of x-ray diffraction pattern. The size of NPs was estimated using Debye-Scherer formula. Figure 6(A) exhibits the XRD pattern of the as-synthesized nanoparticles. Copper nanoparticles exhibited some prominent peaks at 2θ values; 18.8°, 25.8°, 27.02°, 31.2°, 32.63°, 43.8°. Zinc nanoparticles exhibited some prominent peaks at 2θ values; 20.21°, 24.6°, 30.6°, 39.24°, 48.86°. Copper-zinc nanoparticles exhibited some prominent peak values at 2θ values at; 16.01°, 18.41°, 24.02°, 28.02°, 31.03°, 32.83°, 37.04°, 45.25°, 49.46° (Riaz *et al.*, 2022).

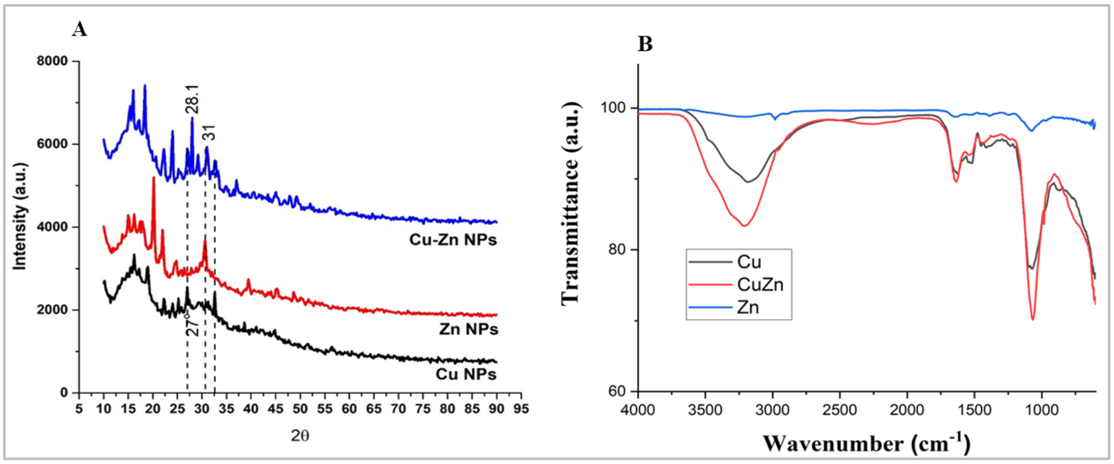


**Fig. 6: Comparative study of (A) XRD of green synthesized Cu, Zn, and Cu-Zn alloy NPs, (B) FTIR of green synthesized Cu, Zn, and Cu-Zn alloy NPs.**

The crystal structure of as-synthesized NPs was studied in terms of an x-ray diffraction pattern. The size of NPs was estimated using the Debye-Scherer formula.

$$\text{Grain size (D)} = \frac{0.9\,\lambda}{\beta\ cos\,\theta}$$

Based on the calculation, the size of copper NPs, zinc nano particles and copper-zinc alloy nanoparticles were found to be 33.87 nm, 29.71 nm and 49.67 nm, respectively.

## Fourier Transform infrared spectroscopic study

The broad peak from 3000 cm$^{-1}$ to 3600 cm$^{-1}$ corresponds to the hydrogen-bonded phenolic group attached to the nanoparticles (Figure 6 B). The peak around 1650 cm$^{-1}$ corresponds to some sort of amide group. The peaks around 1500 cm$^{-1}$ correspond to the C=C of the carbon chain. The absorption peak at 1070 cm$^{-1}$ corresponds to the C-O stretching of primary alcohol.

**Antimicrobial activities of Cu NPs, Zn NPs, and Cu-Zn alloy NPs**

The antimicrobial activities of copper, zinc and copper-zinc nanoparticles synthesized from *Tinospora cordifolia* (Gurjo) stem extract were performed against two American Type Culture Collection (ATCC) and a fungus using Agar well diffusion method, and the results are shown in figure 7.

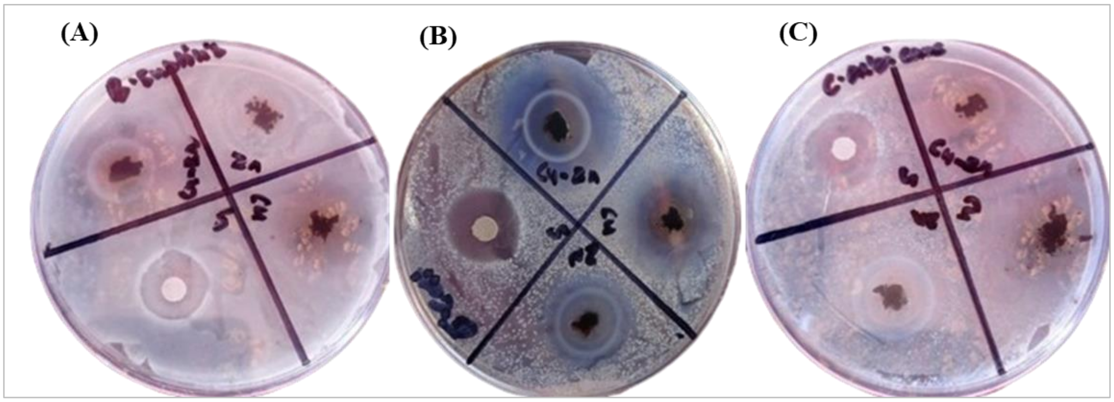


**Fig. 7**: **The antibacterial and antifungal effect exhibited by Cu NPs, Zn NPs, and Cu-Zn alloy NPs against (A) *Bacillus subtilis,* (B) *Escherichia coli* and (C) *Candida albicans.***

The measured value of zone of inhibition has been shown in Table 4.

**Table 4**: The inhibition zone is shown by Cu NPs, Zn NPs, and Cu-Zn alloy NPs

| Test Microorganisms Organism | Reference Culture | Type | List of Nanoparticles | Zone of Inhibition (cm) |
|---|---|---|---|---|
| *Bacillus subtilis* | ATCC 6051 | Gram positive | (a) Cu NPs | 2.4 |
| | | | (b) Zn NPs | 2.3 |
| | | | (c) Cu-Zn alloy NPs | 3.0 |
| *Escherichia coli* | ATCC 8739 | Gram-negative | (a) Cu NPs | 2.7 |
| | | | (b) Zn NPs | 2.9 |
| | | | (c) Cu-Zn alloy NPs | 3.5 |
| *Candida albicans* | ATCC 2091 | _ | (a) Cu NPs | 2.2 |
| | | | (b) Zn NPs | 2.3 |
| | | | (c) Cu-Zn alloy NPs | 2.7 |

Result shows that all the NPs exhibited antibacterial/antifungal activities against *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*. The interesting finding is that the Cu-Zn alloy NPs exhibited the highest zone of inhibition in all cases. It shows the greater effectiveness of Cu-

Zn alloy NPs against the tested micro-organism. As-synthesized alloy NPs exhibited good antimicrobial properties even more effective than that shown by standard *Kanamycin* which could be due to the synergetic effort of copper and zinc nanoparticles. These types of NPs are of great uses for Gram positive, Gram negative as well as fungal strain. Further study can be performed regarding the crux of mechanism.

## 4. Conclusions

The phytochemical screening analysis of the aqueous stem extract of *Tinospora cordifolia* revealed the presence of alkaloids, flavonoids, glycosides, terpenoids, saponins, and quinones. Copper, zinc, and copper-zinc alloy nanoparticles were successfully synthesized using the stem extract of Gurjo. Characterization of the synthesized copper, zinc, and copper-zinc alloy nanoparticles has been successfully done using UV-Vis, FTIR, and XRD techniques. UV-Visible spectroscopy study informed the formation of Cu, Zn, and Cu-Zn alloy NPs. Stabilization of copper, zinc, and copper-zinc alloy NPs is thought to occur due to the assistance of phytochemicals present in the stem extract. Investigations on the antimicrobial effect of the synthesized Cu, Zn, and Cu-Zn alloy NPs were performed against ATCC bacteria (Gram-positive: *Bacillus subtilis* and Gram-negative: *Escherichia coli*) and fungi (*Candida albicans*) which shows satisfactory results. Therefore, this greener approach towards the synthesis of Cu, Zn, and Cu-Zn alloy NPs, using stem-extract as a reducing agent and capping agent can be scaled up with further optimization in the future work.

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**Conflicts of interest:** The authors declare to have no conflict of interest.

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