

## ANTIBACTERIAL ACTIVITIES OF Cu, Ni-DOPED ZnO NANOCOLLOIDS AND ETHIOPIAN TRADITIONAL MEDICINAL PLANT *BERSAMA ABYSSINICA*

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**ABSTRACT.** The present study aimed to evaluate the antibacterial efficacy of pure and doped zinc oxide (ZnO) nanocolloids (Cu and Ni) synthesized via a green chemical method, in comparison with *Bersama abyssinica*, a traditional Ethiopian medicinal plant. The target was to assess their activity against selected Gram-positive and Gram-negative bacterial strains. Pure and doped ZnO nanocolloids were synthesized via a chemical solution method and characterized using XRD, FTIR, UV-Vis, and PL spectroscopy. Their antibacterial activity was evaluated through the agar well diffusion method against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. At 500 mg/mL, *Bersama abyssinica* extract showed good antibacterial activity, especially against *P. aeruginosa* (25.7 mm), *A. baumannii* (19.3 mm), and *S. aureus* (16.4 mm). Doped ZnO nanocolloids performed even better, with inhibition zones up to 28.7 mm. Cu-doped ZnO showed the strongest effect against *S. aureus* (24.2 mm); at the same time, Ni-doped ZnO showed the highest activity on *P. aeruginosa*. However, *E. faecalis* is resistant to plant extract and nanocolloids. Cu and Ni doping improved the antibacterial performance of ZnO nanocolloids. The doped materials were more effective than the plant extract, particularly against Gram-negative strains, suggesting their potential as alternative antimicrobial agents.

**KEY WORDS:** ZnO, Nanocolloids, *Bersama abyssinica*, Gram-negative/positive bacteria, Traditional medicine, Antibacterial effect

## INTRODUCTION

Nanocolloids are colloidal suspensions that consist of nanoparticles typically ranging from 1 to 100 nanometers in size, dispersed in a solvent or medium [1]. These systems often incorporate metals, metal oxides, polymers, or biological molecules, and are distinguished by their unique physicochemical properties, including high surface area, quantum effects, and tunable surface chemistry[2]. Owing to these attributes, nanocolloids have found wide-ranging applications in fields such as biomedicine, environmental remediation, energy conversion and storage, catalysis, sensors, and consumer products [3].

Particularly, colloidal zinc oxide (ZnO) and silver (Ag) nanocolloids have attracted attention for their antimicrobial activity, especially against drug-resistant bacterial strains [4, 5]. Drug-resistant pathogens including species from the genera *Enterococcus*, *Staphylococcus*, and *Streptococcus* are a growing global health concern due to increased mortality, economic burden, and the difficulty of treatment[6]. This resistance is largely attributed to the overuse and misuse of antibiotics in both clinical and agricultural settings [7, 8]. According to the World Health Organization (WHO), antimicrobial resistance (AMR) is among the top 10 global public health threats, with increasing prevalence of infectious diseases being closely linked to the rise in AMR

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[9]. In response, extensive research is underway to identify alternative antimicrobial agents, including plant-based remedies and nanoparticle-based formulations [10, 11].

In Ethiopia, traditional medicine remains widely practiced, with approximately 80% of the population relying on it for primary healthcare, and 95% of these remedies are derived from medicinal plants [12]. These plants are used to manage various health conditions, including cancer, liver, neurological, and infectious diseases [12, 13]. One such plant, *Bersama abyssinica*, locally known as Azamir (Amharic) or Lolichisa (Afan Oromo), is traditionally employed for treating ailments such as abdominal pain, diarrhea, cholera, dysentery, malaria, tumors, and snake bites [14]. Investigating the antimicrobial efficacy of such medicinal plants is crucial in light of increasing drug resistance and limited access to modern therapies.

Recent findings also emphasize the potential of metal and metal oxide nanocolloids, especially Ag and ZnO nanocolloids, for enhanced antimicrobial activity. These nanomaterials demonstrate strong reactive oxygen species (ROS) generation, ion release, and membrane-disruptive actions [15, 16]. Batista *et al.* [17] showed that the antimicrobial efficiency of silver nanocolloids largely depends on the nature of polymeric coatings; for instance, colloids stabilized with PVP and PEO-b-P2VP exhibited significant antibacterial and antifungal activities, while those coated with PEI did not. The ZnO nanomaterials are gaining popularity due to their broad-spectrum activity, low toxicity, excellent biocompatibility, and environmental friendliness [15, 18, 19]. Zakharova *et al.* [19] highlighted that the antibacterial efficacy of ZnO nanoparticles depends on factors such as particle size, aggregation state, storage conditions, and colloidal stability. Despite their effectiveness, ZnO nanocolloids have limitations, including reduced activity under visible light.

Zinc oxide (ZnO) nanoparticles are known to exhibit stronger antibacterial effects against Gram-negative bacteria, such as *Escherichia coli* and *Proteus vulgaris*, than against Gram-positive species, like *Staphylococcus aureus* and *Streptococcus mutans*, primarily due to the structural complexity of the Gram-negative outer membrane [18]. To improve the antimicrobial and photocatalytic efficiency of ZnO across various light conditions, researchers have investigated doping it with metals (e.g., Ag, Cu, Ni), nonmetals, or composite materials [20]. In particular, incorporating transition metals like copper (Cu) and nickel (Ni) into ZnO has shown promising enhancements in antibacterial performance. Copper doping is associated with increased reactive oxygen species (ROS) generation, more oxygen vacancies, and its inherent antimicrobial activity [21]. Meanwhile, nickel enhances ZnO's surface reactivity, improves charge separation, and induces structural changes that collectively contribute to membrane disruption in bacteria [22]. However, despite the growing interest in doped ZnO materials, there is a lack of comprehensive studies investigating their antibacterial effects in colloidal suspension. Moreover, the antimicrobial potential of *B. abyssinica* remains underexplored. To fill these gaps, this study evaluates the antibacterial properties of Cu- and Ni-doped ZnO nanocolloids and compares their efficacy with that of an aqueous extract of *B. abyssinica*. Using the agar well diffusion method, the study examines their activity against both Gram-positive and Gram-negative bacterial strains. The findings aim to bridge traditional plant-based remedies with modern nanomaterials in the ongoing effort to combat antimicrobial resistance.

## MATERIALS AND METHODS

### *Plant collection, identification, and extract preparation*

Fresh leaves of *Bersama abyssinica* were collected from Sebeta, Oromia Region, Ethiopia, situated approximately 25 km southwest of Addis Ababa. The plant was taxonomically identified at the National Herbarium, Department of Plant Biology and Biodiversity Management, College of Natural and Computational Sciences (CNCS), Addis Ababa University (AAU), where a voucher specimen was deposited for future reference. Collected leaves were washed thoroughly with tap water to remove dust and debris, then air-dried at ambient room temperature (22–25 °C)

in a shaded, dust-free environment for 10-15 days. The dried leaves were ground into a fine powder using an electric grinder (Model: RRH 1500A) and stored in amber-colored, airtight glass containers to protect them from moisture and light degradation.

A crude aqueous extract was prepared by macerating 50 g of the powdered leaf material in 500 mL of distilled water (1:10 w/v) in a 1 L Erlenmeyer flask. The mixture was agitated on an orbital shaker (DKZ Series Shaking Water Bath) at 150 rpm for 72 h at room temperature. The extract was filtered using Whatman No. 1 filter paper, and the filtrate was frozen at -20 °C. Freeze-drying was performed using a laboratory lyophilizer (Christ Alpha 2-4 LD Plus) to obtain the dry extract, which was then stored at 4 °C until further use. These protocols are adapted from various references, including [23].

#### *Synthesis of pure ZnO and Cu-, Ni-doped ZnO nanocolloids*

The precursor solution for undoped ZnO nanocolloids was prepared by dissolving 219.5 mg of zinc acetate dihydrate ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ,  $\geq 98\%$ , Sigma-Aldrich) in 2 mL of 2-methoxyethanol ( $\geq 99\%$ , Sigma-Aldrich), followed by the addition of 60  $\mu\text{L}$  of ethanolamine ( $\geq 99\%$ , Sigma-Aldrich) as a stabilizer. The mixture was stirred continuously at 60 °C for 2 h in a closed system using a magnetic stirrer with a hot plate, then allowed to stir in open air for an additional 12 h at room temperature. A clear, transparent gel indicated the successful formation of ZnO colloids. The experiment was done with a slight modification of the method applied by Gómez-Núñez, A. *et al.* [24]. To synthesize metal-doped ZnO nanocolloids, 1-4 wt% of zinc acetate was replaced with nickel nitrate hexahydrate ( $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\geq 98\%$ , Sigma-Aldrich) or copper (II) acetate monohydrate ( $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ ,  $\geq 98\%$ , Sigma-Aldrich). The same procedure was followed as for the undoped ZnO synthesis.

The resulting nanocolloids were characterized to confirm structural and functional properties using the following techniques: X-ray diffraction (XRD) (XRD-7000 X-ray diffractometer, Shimadzu (Japan), Cu-K $\alpha$  radiation,  $\lambda = 1.5406 \text{ \AA}$ ) for phase and crystallite size analysis; Fourier-transform infrared spectroscopy (FT-IR) (Spectrum 65 FT-IR, PerkinElmer) for functional group identification; UV-visible spectroscopy (UV-vis) (Shimadzu UV-1601) for optical absorption and bandgap analysis and Photoluminescence spectroscopy (PL) for defect emission properties.

#### *Antibacterial activity evaluation*

##### *Bacterial strains and inoculum preparation*

The antibacterial activity was tested against four pathogenic bacterial strains: *Staphylococcus aureus* (Gram-positive), *Enterococcus faecalis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Acinetobacter* sp. (Gram-negative). These strains were obtained from the culture collection of the Microbiology and Biomedical Laboratory, Department of Microbial, Cellular and Molecular Biology, CNCS, AAU.

Each strain was sub-cultured on nutrient agar (Oxoid, UK) and incubated at 37 °C for 24 h. A single colony was transferred into 5 mL of nutrient broth and incubated for another 24 h at 37 °C. The bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard (approx.  $1.5 \times 10^8 \text{ CFU/mL}$ ) using sterile saline solution [7].

##### *Antibacterial susceptibility testing*

Antibacterial activity of the extracts and nanocolloids was assessed using the agar well diffusion method. Mueller-Hinton Agar (MHA, Oxoid, UK) plates were prepared and surface-inoculated with 100  $\mu\text{L}$  of the standardized bacterial suspensions using sterile cotton swabs. Wells of 6 mm diameter were made in the agar using a sterile cork borer [25]. Each well was filled with 60-70

$\mu\text{L}$  of the test solutions: Bersama abyssinica aqueous extract, Undoped ZnO nanocolloid, Ni- and Cu-doped ZnO nanocolloids (1-4%), Positive control: Ciprofloxacin (5  $\mu\text{g}/\text{disc}$ , Oxoid), Negative controls: Distilled water and nanocolloid solvent (ethanolamine + 2-methoxyethanol). Plates were incubated at 37 °C for 18-24 h, after which zones of inhibition were measured in millimeters using a digital caliper [7, 25]. Images of inhibition zones were further analyzed using the ImageJ software (NIH, USA) to ensure precision. All tests were carried out in triplicate, and mean values were reported.

## RESULTS AND DISCUSSION

### XRD analysis

Figure 1a presents the X-ray diffraction (XRD) patterns of pure, Ni-doped, and Cu-doped ZnO thin films. The major diffraction peaks observed at  $2\theta$  values of  $31.68^\circ$  (100),  $34.36^\circ$  (002),  $36.22^\circ$  (101),  $47.23^\circ$  (102),  $56.70^\circ$  (110), and  $68.42^\circ$  (112) correspond to the hexagonal wurtzite structure of ZnO, in good agreement with the standard JCPDS card No. 36-1451 [26] and consistent with earlier reports [27]. The sharp and intense nature of these peaks indicates that all samples possess good crystallinity. Upon doping with Ni and Cu, noticeable changes in the diffraction patterns were observed. For the Ni-doped ZnO sample, the (102) peak shifted from  $47.23^\circ$  to  $45.16^\circ$ . These changes suggest distortion of the ZnO lattice due to substitution of  $\text{Zn}^{2+}$  ions (ionic radius 88 pm) with smaller  $\text{Ni}^{2+}$  (83 pm) and  $\text{Cu}^{2+}$  (87 pm) ions.

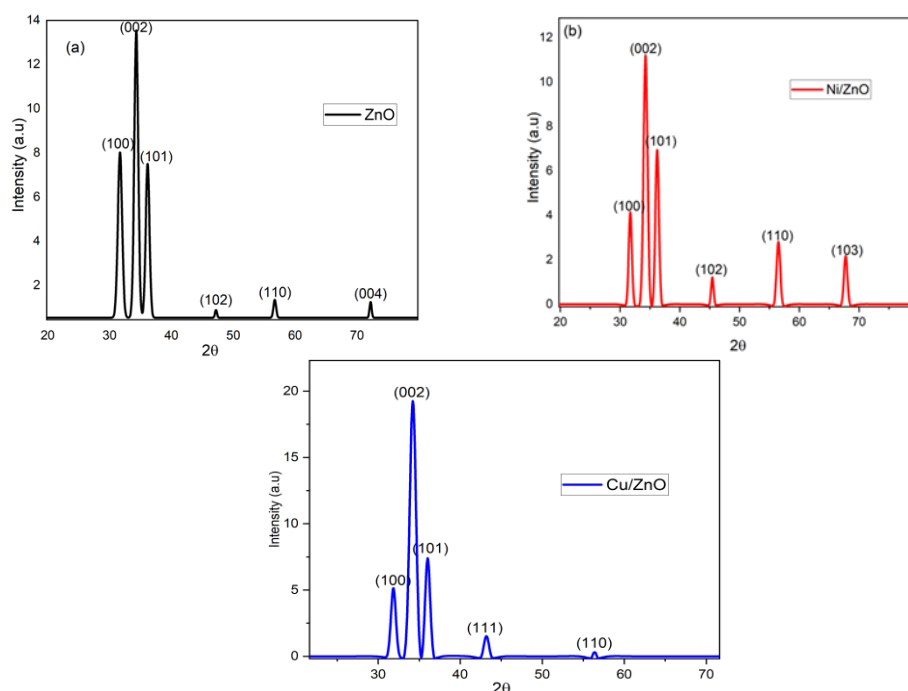


Figure 1. XRD graph of (a) pure ZnO, (b) Ni-doped ZnO, and (c) Cu-doped ZnO.

The XRD pattern of the Cu-doped ZnO nanothin film showed the expected diffraction peaks for the hexagonal wurtzite structure of ZnO, including the main reflections around  $31.7^\circ$ ,  $34.4^\circ$ ,

and  $36.2^\circ$ , which correspond to the (100), (002), and (101) planes, respectively (JCPDS Card No. 36-1451). These peaks confirm that the overall crystal structure of ZnO was retained after doping. Interestingly, an additional peak appeared at around  $43.3^\circ$ , which is not typical for pure ZnO [26]. This peak is likely associated with the (111) plane of either metallic copper (Cu) or copper (I) oxide (CuO), based on standard diffraction data (JCPDS 04-0836 and 05-0667). This suggests that some of the copper introduced during synthesis did not fully substitute into the ZnO lattice. Instead, a portion may have formed a separate phase, either as elemental copper or Cu<sub>2</sub>O. Such secondary phases often emerge when the amount of dopant exceeds the capacity that ZnO can accommodate, or when the synthesis conditions cause partial reduction of Cu<sup>2+</sup> ions. In this case, the presence of the  $43.3^\circ$  peak points to partial segregation of copper, indicating that not all of it was incorporated into the ZnO crystal structure as intended. The formation of separate Cu or Cu<sub>2</sub>O phases isn't necessarily a drawback; it may even enhance the antibacterial properties, as both have their own antimicrobial activity. However, it does mean the doping process was not entirely uniform, which could affect properties like optical clarity or electrical behavior.

The average crystallite sizes were estimated using Scherrer's equation (Equation 1) [27]. The pure ZnO sample showed an average size of 14.78 nm, while Ni-doped and Cu-doped samples exhibited smaller crystallite sizes of 13.08 nm and 11.45 nm, respectively. The reduction in crystallite size upon doping is likely due to lattice strain and defects introduced by ionic substitution, which can influence the material's optical, electrical, and surface properties [21].

$$D = \frac{k\lambda}{\beta \cos \theta} \quad 1$$

Where  $D$  indicates the average crystal size,  $k$  indicates the Scherrer constant, which depends on the wurtzite crystal shape,  $\lambda$  is the wavelength of Cu-K $\alpha$  X-ray radiation,  $\beta$  is the full width at half maximum of XRD peaks and  $\theta$  is the diffraction angle. These results are in good agreement with previously reported findings [26, 28-30], validating the successful synthesis of nanocrystalline doped ZnO thin films with uniform morphology and high structural quality.

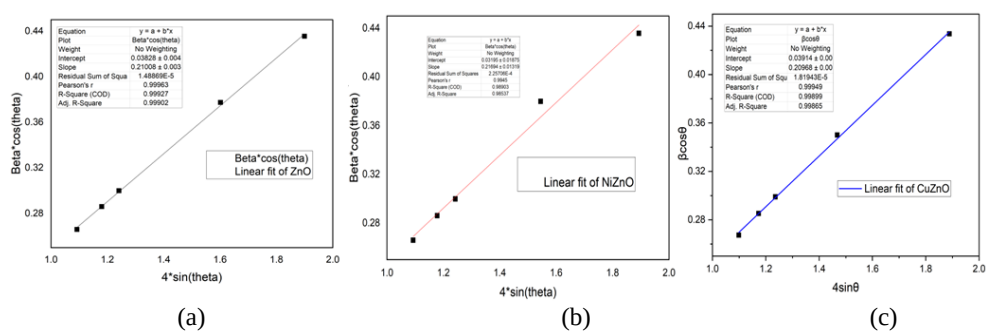


Figure 2. Williamson-Hall plot of (a) undoped ZnO, (b) NiZnO, and (c) CuZnO.

Figure 2 shows that the lattice strain of ZnO, Ni-doped ZnO, and Cu-doped ZnO nanocolloids, calculated using the Williamson–Hall (W–H) method, was found to be 0.0525, 0.0542, and 0.0524, respectively. Although the differences appear minor, such strain variations significantly impact antibacterial behavior. Lattice strain indicates internal distortion within the crystal structure, often induced by doping. This distortion promotes the formation of oxygen vacancies and surface defects, which enhance reactive oxygen species (ROS) production, a primary mechanism through which ZnO-based nanomaterials inhibit bacterial growth.

Among the three, Ni-doped ZnO, which had the highest strain, showed superior antibacterial activity, likely due to the increased density of surface defects and improved ROS generation. Similarly, Cu-doped ZnO also showed better antibacterial performance than undoped ZnO, benefiting from both lattice strain and the antimicrobial nature of  $\text{Cu}^{2+}$  ions. In summary, the results suggest that even slight increases in lattice strain, especially when combined with metal ion doping, can enhance the antibacterial efficiency of ZnO nanocolloids by amplifying ROS-related mechanisms [21].

#### FTIR analysis

The FTIR spectral studies of pure ZnO, Cu-doped ZnO, and Ni-doped ZnO nano-colloids were conducted to investigate the purity and characteristics of metal nanoparticles in the colloids. This spectrum illustrates IR absorption resulting from different vibration modes. Typically, metal oxides exhibit absorption bands in the fingerprint region, specifically below  $1000\text{ cm}^{-1}$  which are attributed to inter-atomic vibrations [4, 12]. Figure 2 displayed the series of absorption peaks that correspond to the impurities found in the samples. The broad observed around  $3395.2$  and  $1124.8\text{ cm}^{-1}$  may be due to O-H stretching and deformation, which may be assigned to the water adsorption on the metal surface [12, 30]. The peaks around  $2850\text{--}3000\text{ cm}^{-1}$  reveal its C-H stretching vibrations, and the C-H bending vibration of alkane is observed around  $1383\text{ cm}^{-1}$  and  $1460\text{ cm}^{-1}$ . Also, C-O stretching (acid) observed at  $1280\text{ cm}^{-1}$  and  $1330\text{ cm}^{-1}$  [26]. The prominent peaks at  $1068\text{ cm}^{-1}$  and  $1195.3\text{ cm}^{-1}$  are due to the C-O stretch (alcohol). The band at  $11585\text{ cm}^{-1}$  is due to the C=C stretch, while the region  $832\text{--}1000\text{ cm}^{-1}$  is due to the =C-H bending of alkene. The peak at  $672\text{ cm}^{-1}$  is attributed to O=C=O bending vibration [26]. The peaks at  $617.5\text{ cm}^{-1}$ ,  $537.4\text{ cm}^{-1}$  and  $460\text{ cm}^{-1}$  may correspond to Zn-O, Ni-O, and Cu-O stretching and deformation vibration. The FTIR spectral result matches with previous research findings for undoped and Ni, Cu doped zinc oxide nanoparticles [4, 12, 30, 31].

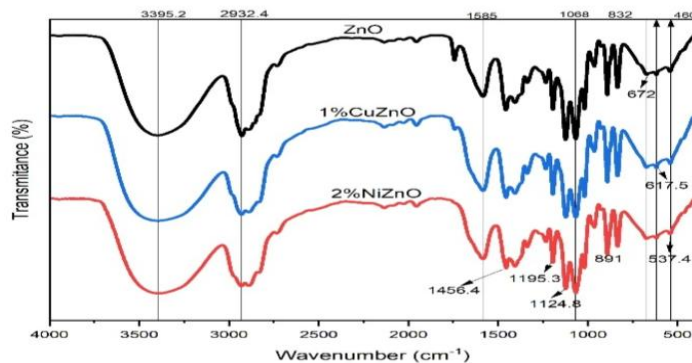


Figure 3. FTIR graph of undoped and Ni, Cu-doped ZnO.

#### UV-Vis spectroscopy analysis

The UV-Vis absorbance of synthesized undoped, Ni, and Cu-doped ZnO nanocolloids was measured within  $200\text{--}1100\text{ nm}$  wavelength. Figure 3 shows the presence of spectral peaks in the UV-Vis spectra at  $340\text{ nm}$ , which indicates the successful synthesis of undoped ZnO, and we observe a slight difference for Ni, Cu-doped ZnO nanocolloids, respectively. When comparing undoped ZnO nanocolloids with Ni, Cu-doped ZnO nanocolloids, the absorption edge of the doped ZnO was slightly red-shifted toward higher wavelength. This shift in absorption edge could

be attributed to a decrease in the band gap and its values are calculated using Stern relation as indicated in (Eq.2) [27]. As a result, we obtained that the energy band gap of pure, Ni, Cu doped ZnO nanocolloids are 3.29 eV, 3.27 eV and 3.25 eV, respectively. The result is in good agreement with findings of previous studies [30]. The obtained energy band gap values of Cu- and Ni-doped ZnO show a red shift compared with pure ZnO (3.37 eV) and bulk ZnO [32].

$$A h \nu = k(h \nu - E_g)^n \quad 2$$

Where k is a constant, h is the Planck's constant,  $\nu$  is the photon frequency,  $E_g$  is the band gap energy, A is the absorption coefficient, and n is a characteristic of the optical absorption process (i.e.,  $n = 1/2$  and  $n = 2$  for direct band gap semiconductor material and indirect transition, respectively).

These results indicate the successful incorporation of Ni and Cu ions into the ZnO lattice crystals, which results in an increased capacity for capturing light due to the reduction of band gap energy and broadening of the absorption band.

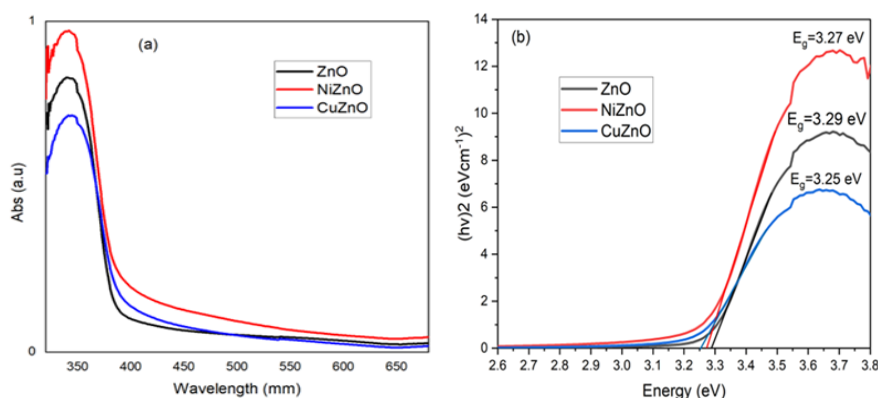


Figure 4. The graph of (a) Uv-Vis spectra, and (b)  $E_g$  of undoped ZnO, Ni, and Cu-doped ZnO.

#### Photoluminescence analysis

Photoluminescence (PL) spectroscopy revealed that ZnO has a significant number of host defects such as zinc vacancies ( $V_{Zn}$ ), zinc interstitials ( $Zn_i$ ), oxygen vacancies ( $V_o$ ), and oxygen interstitials ( $O_i$ ) [33]. Figure 4 displays the photoluminescence profile discussing the Cu, Ni-doped, and undoped ZnO emission spectrum. ZnO and Cu-doped ZnO were excited at a 340 nm wavelength. The peak intensity of the undoped ZnO PL spectra was observed at 511 nm, which is associated with the visible spectrum's blue emission. This emission is caused by point defects that may arise from zinc interstitial ( $Zn_i$ ). The peak intensity for Ni-doped ZnO and Cu-doped ZnO PL spectra was observed at 504 nm and 502 nm, respectively. These are defects due to the dopants, which correspond to the near band gap excitonic emission. Whereas the other emission peak is at approximately 710 nm, which is associated with the presence of singly ionized oxygen vacancies. A photogenerated hole with an electron occupying the oxygen vacancy undergoes radiative recombination, which results in the emission [33]. The outcomes agree well with the findings of previous research [34, 35]. According to the literature, the primary cause of different peaks is a different degree of bonding brought about by the incorporation of various materials (carriers) during the transition process.

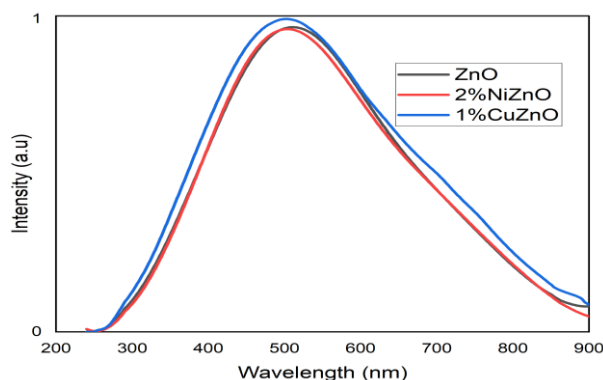


Figure 5. PL graph of undoped ZnO and Cu, Ni-doped ZnO nanocolloids.

#### Antimicrobial studies

There are three mechanisms that can explain the antibacterial effect of Cu, Ni-doped, or undoped ZnO nanocolloids [36]. (1) Physical Contact with cells, nanoparticles have a high surface area to volume ratio that leads them to be very reactive with matter, hence nanoparticles in nanocolloids react with bacterial cells when they are in surface contact with the microbial cells due to the electrostatic force, as shown in Figure 5. Resulting the cell wall being ruptured and the cell membrane [36, 37]. (2) The dissolution of  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ , or  $\text{Zn}^{2+}$  released from Cu, Ni-doped or undoped ZnO nanocolloids. These released ions are adsorbed by the surface of negatively charged bacterial cells due to electrostatic interaction; as a result, the microorganism growth can be interrupted since the synthesis and metabolism of proteins are inhibited by the released ions. The source of negative charge is primarily due to the presence of negatively charged components in the cell wall, such as teichoic acids in Gram-positive bacteria and lipopolysaccharides (LPS) in Gram-positive and Gram-negative bacteria, respectively [38].

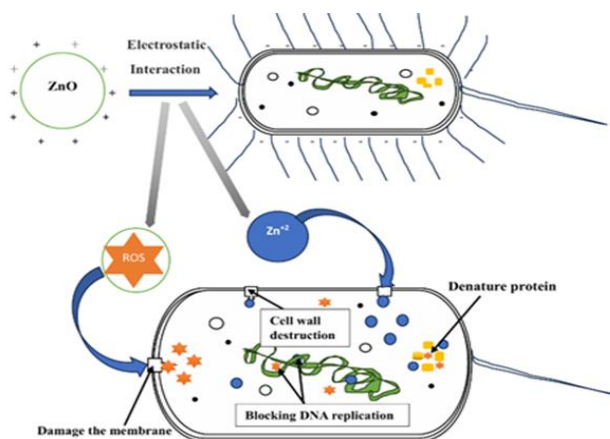


Figure 6. Schematic illustration of the mechanisms underlying antibacterial activity.

Ion concentrations above 50 mg/L would disrupt the enzyme activity of nitrifying bacteria and halt the nitrification process [39]. (3) Reactive oxygen species (ROS) are produced by ZnO NPs,

which have optical properties. Bacterial oxidative stress and loss of enzymatic activity nanoparticles are just two of the permanent harms that can arise from the ROS generated by illumination [37].

*Antibacterial activity (zone of inhibition, mm) of B. abyssinica leaf extract at different concentrations*

The present study evaluated the antibacterial activities of *Bersama abyssinica* leaf extract, undoped ZnO nanocolloids, and Cu- and Ni-doped ZnO nanocolloids against selected Gram-positive and Gram-negative pathogens.



I. (a) Cu/ZnO

(b) *B. abyssinica*

(c) Ni/ZnO



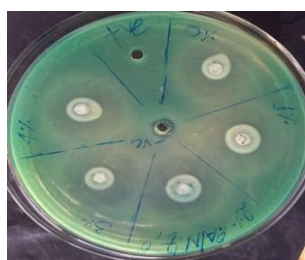
II. (a) Cu/ZnO

(b) *B. abyssinica*

(c) Ni/ZnO



III. a) Cu/ZnO

(b) *B. abyssinica*

(c) Ni/ZnO

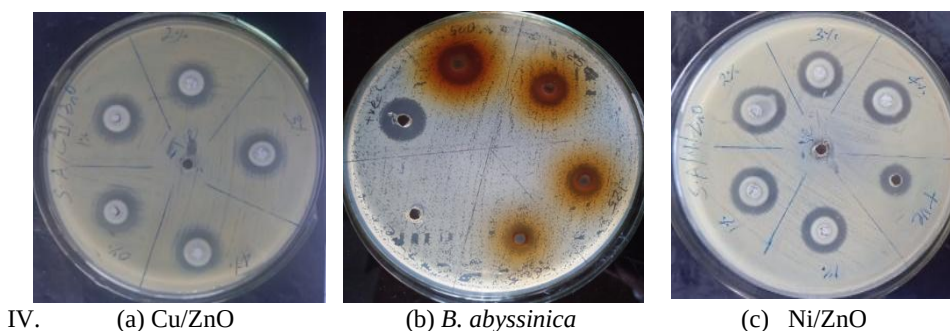


Figure 7. Zone of inhibition for *B. abyssinica*, NZO, CZO and pure ZnO against (i) against *Acinetobacter*, (ii) *E. faecalis*, (iii) *P. aeruginosa*, and (iv) *S. aureus*.

The findings revealed that all agents exhibited inhibitory effects with varying degrees of efficacy depending on bacterial species, concentration, and dopant type, as shown in Figure 7.

#### Antibacterial activity of *Bersama abyssinica* extract

Table 1. Antibacterial Activity (Zone of Inhibition, mm) of *B. abyssinica* Leaf Extract Against Selected Pathogens.

| Concentration (mg/mL) | Study                   | <i>S. aureus</i> | <i>E. faecalis</i> | <i>P. aeruginosa</i> | <i>A. baumannii</i> | Notes                |
|-----------------------|-------------------------|------------------|--------------------|----------------------|---------------------|----------------------|
| 500                   | This study (2025)       | 16.4             | –                  | 25.7                 | 19.2                | Aqueous leaf extract |
| 250                   | This study (2025)       | 12.6             | –                  | 21.3                 | 17.2                |                      |
| 100                   | Anza, M. (2023)         | 11               | -                  | -                    | -                   | Aqueous extract      |
| 125                   | This study (2025)       | 11.2             | –                  | 16.9                 | 15.5                |                      |
| 62.5                  | This study (2025)       | 9.7              | –                  | 12.3                 | 10.6                |                      |
| –                     | Ciprofloxacin (Control) | 12.0             | 12.6               | 23.7                 | 1.6                 | Standard antibiotic  |

The antibacterial performance of *Bersama abyssinica* leaf extract was evaluated against both Gram-positive and Gram-negative bacterial strains at four different concentrations. The results indicate that the extract exhibits concentration-dependent inhibitory activity, with larger zones of inhibition observed at higher concentrations, especially against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as indicated in table 1. At 500 mg/mL, *B. abyssinica* demonstrated a strong inhibitory effect against *P. aeruginosa* (25.7 mm) and *A. baumannii* (19.2 mm), while *S. aureus* showed a slightly lower response (16.4 mm). No detectable inhibition was observed against *Enterococcus faecalis* across all tested concentrations. These results suggest that *B. abyssinica* is particularly effective against Gram-negative bacteria, which are often more resistant to plant-based antimicrobials due to their complex outer membranes.

Comparing these findings to previous studies, the extract in this study shows comparable or enhanced activity. For instance, at 100 mg/mL, Anza, M. (2023) [40] reported inhibition zones of 11 mm for similar pathogens using an aqueous extract. The stronger performance in the present study, especially at 500 mg/mL, may be attributed to differences in the extraction solvent, plant source variability, or bacterial strain sensitivity. The aqueous extract used here retains significant bioactivity, indicating that water-soluble phytochemicals in *B. abyssinica* contribute substantially to its antimicrobial effects.

Notably, *B. abyssinica* extract demonstrated greater inhibition against *P. aeruginosa* than the standard antibiotic ciprofloxacin (25.7 mm vs. 23.7 mm), underscoring its potential as a natural alternative or adjunct treatment. However, the activity against *S. aureus* was slightly lower than ciprofloxacin (16.4 mm vs. 12.0 mm), and significantly lower against *E. faecalis*, where no inhibition was recorded. This suggests selectivity in antimicrobial action, which may be advantageous in avoiding disruption of beneficial microbiota. The absence of activity against *E. faecalis* raises interesting questions regarding the composition of the extract and its mode of action. It is possible that the bioactive components lack sufficient membrane permeability or specific targeting mechanisms required to inhibit this species. Further phytochemical profiling and mechanistic studies are needed to understand these variations. In general, *B. abyssinica* leaf extract exhibits promising antibacterial activity, particularly against Gram-negative pathogens at higher concentrations. These results support its traditional medicinal use and highlight its potential as a source of natural antimicrobial agents. The observed concentration-dependent trend reinforces the need for standardized dosing in future applications.

#### Enhanced activity of doped ZnO nanocolloids

This study examined the antibacterial properties of Cu- and Ni-doped ZnO nanocolloids synthesized through a chemical solution method and evaluated their activity against four clinically relevant bacterial strains.

Table 2. Zone of inhibition (mm) demonstrating the antibacterial activity of undoped, Cu-doped, and Ni-doped ZnO nanocolloids against selected pathogens.

| Study                       | Dopant | Conc. (%) | <i>S. aureus</i> | <i>P. aeruginosa</i> | <i>E. faecalis</i> | <i>A. baumannii</i> | Notes                                                |
|-----------------------------|--------|-----------|------------------|----------------------|--------------------|---------------------|------------------------------------------------------|
| This study (2025)           | Cu     | 0         | 20.5             | 24.0                 | –                  | 17.9                | Undoped ZnO (chemical synthesis)                     |
|                             | Cu     | 1         | 24.2             | 26.7                 | –                  | 20.1                |                                                      |
|                             | Cu     | 2         | 20.6             | 24.6                 | –                  | 18.2                |                                                      |
|                             | Cu     | 3         | 22.3             | 23.5                 | –                  | 19.0                |                                                      |
|                             | Cu     | 4         | 22.7             | 24.0                 | –                  | 19.1                |                                                      |
| This study (2025)           | Ni     | 1         | 18.9             | 28.7                 | –                  | 18.5                | Ni-doped ZnO (chemical synthesis)                    |
|                             | Ni     | 2         | 19.0             | 26.2                 | –                  | 19.6                |                                                      |
|                             | Ni     | 3         | 17.8             | 25.7                 | –                  | 19.0                |                                                      |
|                             | Ni     | 4         | 17.4             | 27.5                 | –                  | 18.3                |                                                      |
| Naskar <i>et al.</i> (2020) | Ni     | 0         | –                | –                    | –                  | –                   | Lower than 2NZO and 5NZO                             |
| Naskar <i>et al.</i> (2020) | Ni     | 2         | –                | –                    | –                  | –                   | Reported 15-18 mm ZOI for <i>E. coli</i> (not shown) |
| Naskar <i>et al.</i> (2020) | Ni     | 5         | –                | –                    | –                  | 22                  |                                                      |
| Ciprofloxacin (control)     | –      | –         | 12.0             | 23.7                 | 12.6               | 9.9                 | Standard antibiotic                                  |

As indicated in Table 2, the results show a clear enhancement in antibacterial activity following metal ion doping, with notable strain-specific responses and dose-dependent effects. For Cu-doped ZnO, the inhibition zone for *Staphylococcus aureus* increased from 20.5 mm for the undoped sample to a peak of 24.2 mm at 1% doping, indicating improved activity even at low dopant levels. A similar trend was observed for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, with maximum inhibition zones of 26.7 mm and 20.1 mm, respectively, also at 1% Cu. Interestingly, further increases in Cu concentration beyond 1% did not result in a consistent rise in inhibition, suggesting that optimal antibacterial performance occurs at moderate doping

levels, possibly due to a balance between defect generation and particle agglomeration. Ni-doped ZnO nanocolloids also showed improved antibacterial activity, particularly against *P. aeruginosa*, which reached a maximum inhibition zone of 28.7 mm at 1% Ni. *A. baumannii* showed a modest but consistent response, ranging from 18.3 mm to 19.6 mm across 1-4% doping. The slight drop in activity for *S. aureus* at higher Ni concentrations suggests that Ni incorporation can influence surface reactivity in complex ways, potentially altering charge transfer dynamics or particle morphology. The *E. abyssinica*-mediated synthesis reported in 2023 yielded a comparable inhibition zone of 20 mm for *S. aureus* at 4% Ni, aligning with the values observed in this study. In general, both Cu and Ni doping enhanced the antimicrobial performance of ZnO nanocolloids compared to the undoped material. This result confirmed the result reported by Naskar *et al.* (2020) [22, 41]. Notably, the doped nanocolloids surpassed ciprofloxacin, the positive control, in their activity against *P. aeruginosa*, which is a notoriously resistant Gram-negative pathogen. While ciprofloxacin showed a 23.7 mm inhibition zone, Cu- and Ni-doped ZnO reached up to 26.7 mm and 28.7 mm, respectively. However, neither the doped nor the undoped nanocolloids showed inhibitory effects against *Enterococcus faecalis*, suggesting that additional functionalization or a synergistic agent might be needed to extend their antibacterial spectrum.

The observed improvements can be attributed to several mechanisms, including increased generation of reactive oxygen species (ROS), enhanced surface defect density, and better particle–cell membrane interactions due to the presence of transition metal dopants. These modifications likely disrupt cellular respiration, induce oxidative stress, and compromise membrane integrity in susceptible bacterial strains. The results confirm that metal ion doping, particularly at low to moderate concentrations, significantly enhances the antibacterial effectiveness of ZnO nanocolloids. These findings support the development of doped nanomaterials as eco-friendly and potent alternatives or complements to conventional antibiotics, especially in the era of increasing antimicrobial resistance. The comparison between plant extract and nanocolloids is tabulated in Table 3.

Table 3. Antibacterial activity (zone of inhibition, mm) of *B. abyssinica* extract, undoped and doped ZnO nanocolloids.

| Material / Agent                         | <i>S. aureus</i> | <i>P. aeruginosa</i> | <i>A. baumannii</i> | Notes                              |
|------------------------------------------|------------------|----------------------|---------------------|------------------------------------|
| <i>B. abyssinica</i> extract (500 mg/mL) | 16.4             | 25.7                 | 19.2                | Aqueous leaf extract               |
| Undoped ZnO                              | 20.5             | 24.0                 | 17.9                | Chemically synthesized ZnO         |
| Cu-doped ZnO (1%)                        | 24.2             | 26.7                 | 20.1                | Chemically synthesized nanocolloid |
| Ni-doped ZnO (1%)                        | 18.9             | 28.7                 | 18.5                | Chemically synthesized nanocolloid |
| Ciprofloxacin (control)                  | 12.0             | 23.7                 | 9.9                 | Standard antibiotic                |

The antibacterial activities of *Bersama abyssinica* leaf extract, undoped ZnO nanocolloids, and Cu- and Ni-doped ZnO nanocolloids were evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The inhibition zone diameters (mm) are summarized in Table 3. The aqueous leaf extract of *B. abyssinica* exhibited considerable antibacterial activity, with inhibition zones of 16.4, 25.7, and 19.2 mm against *S. aureus*, *P. aeruginosa*, and *A. baumannii*, respectively. This confirms the presence of bioactive phytochemicals with antimicrobial properties.

The undoped ZnO nanocolloid demonstrated enhanced antibacterial effects compared to the plant extract for *S. aureus* (20.5 mm) and comparable activity for *P. aeruginosa* (24.0 mm), though slightly lower activity against *A. baumannii* (17.9 mm). This is attributed to ZnO's known generation of reactive oxygen species (ROS) and disruption of bacterial membranes. Doping ZnO with Cu and Ni improved antibacterial efficacy. Cu-doped ZnO showed the highest inhibition

zones for *S. aureus* (24.2 mm) and *A. baumannii* (20.1 mm), while Ni-doped ZnO was most effective against *P. aeruginosa* (28.7 mm). The increased activity is likely due to dopant-induced lattice defects enhancing ROS production and bacterial cell damage. Moreover, in a Cu doped ZnO nanocolloid, the bacterial activity was highest compared with other samples. This may be due to the antibacterial nature of the Cu atom or its oxides because our XRD result revealed that there is metallic Cu or CuO, which can maximize the antibacterial activity.

Notably, all tested agents outperformed the standard antibiotic ciprofloxacin, which showed inhibition zones of 12.0, 23.7, and 9.9 mm for *S. aureus*, *P. aeruginosa*, and *A. baumannii*, respectively, this indicate that different bacterial strains become resistant to standard antibiotic as mentioned by the WHO [9]. Hence, suggesting the potential of ZnO nanocolloids and plant extracts as alternative antimicrobial agents. These findings indicate a promising synergistic antibacterial effect when combining plant extracts with doped ZnO nanocolloids, offering a potential strategy to address antibiotic resistance.

#### *Mechanisms of antibacterial action*

The improved antimicrobial activity is attributed to several mechanisms. First, UV-Vis and photoluminescence (PL) data confirmed the substitution of  $\text{Zn}^{2+}$  ions by  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  in the ZnO lattice, facilitating the release of toxic metal ions [42]. These ions interfere with bacterial metabolic pathways, DNA structure, and protein function. Secondly, dopants alter surface charge, enhancing interaction with negatively charged bacterial membranes and promoting ROS generation, which damages lipids, proteins, and nucleic acids [43, 44].

ROS-mediated oxidative stress and electrostatic interactions contribute synergistically to bacterial membrane disruption. Additionally, the surface charge modulation caused by different dopants influences nanoparticle–cell interactions, consistent with changes observed in PL spectra. The differences in antibacterial behavior at various dopant levels (1–4%) suggest that moderate doping achieves optimal surface reactivity and defect structure for microbial inhibition.

#### *Gram-negative vs. Gram-positive susceptibility*

Gram-negative bacteria were more susceptible to the tested agents than Gram-positive strains, consistent with earlier reports [45]. This difference stems from the thinner peptidoglycan layer and presence of negatively charged LPS in Gram-negative cells, which favors nanoparticle uptake and ROS accumulation. In contrast, Gram-positive bacteria possess thicker cell walls and higher peptidoglycan content, limiting nanoparticle penetration [46, 47]. Furthermore, positively charged metal ions preferentially interact with LPS-enriched regions of Gram-negative bacteria, enhancing localized toxicity [21]. Both *B. abyssinica* extract and doped ZnO nanocolloids demonstrated promising antibacterial activity, especially against Gram-negative pathogens. The incorporation of Cu and Ni into ZnO significantly enhanced efficacy, surpassing the undoped ZnO and the standard antibiotic in most cases. These results underscore the potential of combining plant-based and nanomaterial-based antimicrobials as alternative strategies to address the growing challenge of antibiotic resistance.

## CONCLUSION

Metal-doped and undoped ZnO nanocolloids were synthesized through the easiest and most cost-effective solution method, and the characterization was performed using XRD, UV-Vis, PL, and FT-IR. Agar well diffusion is used to study antimicrobial activity. This study asserted that the nanocolloids used in this study exhibits hexagonal wurtzite crystal structure. From the UV-Vis spectroscopy, the absorption peak observed in each spectrum within the range of 340–345 nm is indicative of a distinctive band associated with pure ZnO. The energy band gap was obtained as 3.29 eV for undoped ZnO, with a small difference in Ni and Cu-doped ZnO, respectively, at room

temperature. From FTIR, the stretching and bending were found to be 460, 537.4, 612.8  $\text{cm}^{-1}$ , which proved to be Ni-O, Cu-O, and Zn-O Stretching. The particle size and structure were determined through X-ray diffraction, and the obtained values correspond with the reference data. Additionally, the antibacterial activity of the undoped ZnO and Ni, Cu doped ZnO nanocolloids was examined against *E. faecalis*, *Acinetobacter*, *P. aeruginosa*, and *S. aureus*. The result showed that Ni-doped ZnO nanoparticles have a slightly better antibacterial activity except *Acinetobacter* and *E. faecalis* compared with other synthesized nanocolloids as well as *B. abyssinica* extract. However, the aqueous extract of *B. abyssinica* had better antibacterial activity on *Acinetobacter* sp. when compared to nanocolloids and the positive control. Besides, *E. faecalis* was highly resistant to the nanocolloids as well as the plant extract; hence, it needs further investigation.

#### Conflict of interest statement

There is no conflict of interest among the authors.

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