

Original article

## Biogenic synthesis of silver-functionalized iron and copper oxide nanocomposites using the aqueous extract of *Psidium guajava* fruit

### Síntesis biogénica de nanocompuestos de óxidos de cobre y de hierro funcionalizados con plata utilizando el extracto acuoso del fruto de *Psidium guajava*

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## Abstract

Here, we present a green coprecipitation synthesis route for silver-functionalized copper and iron oxide nanocomposites ( $\text{Cu}_2\text{O}/\text{CuO}/\text{Fe}_2\text{O}_3@Ag$ ) using guava (*Psidium guajava*) fruit extract as reducing and stabilizing agent. The novelty of this approach lies in the unexplored use of guava, a widely available biomass rich in phytochemicals, to produce multifunctional nanocomposites with enhanced properties. The structural and morphological analyses (XRD, FTIR, SEM, TEM) confirmed the presence of crystalline phases of  $\text{Fe}_2\text{O}_3$ ,  $\text{Cu}_2\text{O}$ ,  $\text{CuO}$ , and  $\text{Ag}$ , along with nanoflake-like and sphericle-like morphologies and nanometric particle sizes. Magnetic characterization (VSM) revealed a change from paramagnetic behavior in non-functionalized samples to weak ferromagnetism with reduced coercivity and magnetization upon silver incorporation. The antibacterial assays demonstrated that the functionalized nanocomposites exhibited strong inhibitory effects against the multidrug-resistant pathogen *Klebsiella pneumoniae*, achieving substantial growth inhibition at 500 ppm. Our findings highlight the potential of guava fruit extract as a sustainable precursor for the biogenic synthesis of silver-functionalized Fe–Cu oxide nanocomposites, offering a promising route for environmentally friendly nanomaterials with applications in biomedical and environmental fields.

**Keywords:** Green chemistry; Nanomaterials; Copper oxide; Iron oxide; Guava fruit extract; Antibacterial.

## Resumen

Presentamos aquí una ruta verde de síntesis por coprecipitación de nanocompuestos de óxidos de cobre y de hierro funcionalizados con plata ( $\text{Cu}_2\text{O}/\text{CuO}/\text{Fe}_2\text{O}_3@Ag$ ) utilizando extracto de guayaba (*Psidium guajava*) como agente reductor y estabilizador. La novedad de este enfoque radica en el uso inexplorado de la guayaba, una biomasa rica en fitoquímicos ampliamente disponible, para producir nanocompuestos multifuncionales con propiedades mejoradas. Los análisis estructurales y morfológicos (XRD, FTIR, SEM, TEM) confirmaron la presencia de fases cristalinas de  $\text{Fe}_2\text{O}_3$ ,  $\text{Cu}_2\text{O}$ ,  $\text{CuO}$  y  $\text{Ag}$ , además de morfologías similares a nanoescamas y tamaños de partícula nanométricos. La caracterización magnética (VSM) reveló el cambio del comportamiento paramagnético en muestras no funcionalizadas a un ferromagnetismo débil con coercitividad y magnetización reducidas tras la incorporación de plata. Los ensayos antibacterianos demostraron que los nanocompuestos funcionalizados exhibían fuertes efectos inhibidores contra el patógeno resistente a múltiples

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fármacos *Klebsiella pneumoniae*, logrando una inhibición sustancial del crecimiento a 500 ppm. Estos hallazgos destacan el potencial del extracto de la guayaba como precursor sostenible para la síntesis biogénica de nanocompuestos de los óxidos de Fe y Cu funcionalizados con plata, lo que ofrece una ruta prometedora para la obtención de nanomateriales respetuosos del medio ambiente con aplicaciones en campos biomédicos y ambientales.

**Palabras clave:** Química verde; Nanomateriales: óxido de cobre; Óxido de hierro; Extracto de guayaba; Antibacteriano.

## Introduction

Nanomaterials (NM) have gained significant attention in recent decades due to their wide range of applications, including environmental remediation (Kumar *et al.*, 2022), renewable energies (Akin *et al.*, 2019), biomedicine (Naik *et al.*, 2023), and industries (Paidari & Ibrahim, 2021), among others. The vast spectrum of NM uses arises from the unique properties they exhibit; some of their most notable characteristics include a high surface area-volume ratio (Altammar, 2023), ferrimagnetism (Zahn *et al.*, 2022), and ferromagnetic and superparamagnetic behaviors (Rashid *et al.*, 2022). This type of material has also shown promising properties in diagnosing and treating diseases such as cancer (Jose *et al.*, 2020), serving as contrast agents in magnetic resonance imaging, drug delivery (El-Boubbou *et al.*, 2016), and bactericidal applications (Yang *et al.*, 2021). In this context, researchers have studied the biological and bactericidal properties of copper oxide and iron oxide nanostructures (Bhavyasree & Xavier, 2020; Jamzad & Bidkorpeh, 2020; Sharma *et al.*, 2020), finding that specific iron oxide nanostructures like hematite (Fe<sub>2</sub>O<sub>3</sub>) exhibit high antimicrobial activity against bacteria such as *Pseudomonas aeruginosa* (Yoonus *et al.*, 2021). Copper oxide nanoparticles, such as Cu<sub>2</sub>O and delafossite (CuFeO<sub>2</sub>), have also demonstrated notable antibacterial properties against various Gram-positive bacteria strains (*Bacillus subtilis*, *B. cereus*, and *Staphylococcus aureus*) and Gram-negative strains (*Escherichia coli* and *Xanthomonas campestris*) (Antonoglou *et al.*, 2019).

Although copper and iron nano-oxides have shown bactericidal properties, they can be optimized by functionalizing their surfaces (Yoonus *et al.*, 2021) using elements with appreciable antimicrobial qualities. Several metallic nanoparticles (NPs) exhibit these properties. Silver nanoparticles (Ag-NPs), for example, show high toxicity against a variety of microorganisms (Parvekar *et al.*, 2020; Salem *et al.*, 2020). These results suggest that the potential synthesis of a nanocomposite system integrating copper and iron oxides, functionalized with silver, may enhance bacteriostatic and bactericidal properties compared to their non-functionalized counterparts (Antonoglou *et al.*, 2019).

On the other hand, the biogenic approach in NM synthesis has gained great relevance. In this regard, plants have been an ideal and abundant source of biomass useful in the synthesis, since the phytochemicals present in plant extracts can act as reducing and stabilizing agents of inorganic metal ions in NPs synthesis (Chokkareddy & Redhi, 2018). Several studies account for the use of plants' essential oils, fruits, roots, flowers, leaves, and stems to obtain NM (Fakhari *et al.*, 2019; Jadoun *et al.*, 2021; Marslin *et al.*, 2018; Charbgoon *et al.*, 2017; Arsiya *et al.*, 2017; Narayanan *et al.*, 2021; Reddy, 2017; Ismail, 2020; Ramola *et al.*, 2019; Tamoradi & Mousavi, 2020; Cahyana *et al.*, 2021; Aksu Demirezen *et al.*, 2019; Jacob *et al.*, 2017; Veisi *et al.*, 2021; Buarki *et al.*, 2022). In this context, Boyacá is one of the Colombian departments with the highest agrobiodiversity and agricultural production, producing 26,000 tons of vegetables and 98,000 tons of fruits in 2009 (Ayala, 2017), which shows tremendous possibilities given the availability of biological substances that may be used in this synthesis route. The antioxidant properties of guava have been widely evidenced in various studies using DPPH free radical scavenging tests (Manikandan & Anand, 2015). Its extracts have also shown inhibitory properties against several bacteria, among them, *Listeria monocytogenes* and *S. aureus* (Mahfuzul Hoque *et al.*, 2007). Ripe guava fruits are rich in tannins, polyphenols with antioxidant properties, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, fatty acids, fiber, and vitamins. Besides its high

concentrations of vitamin C, higher than those of citrus fruits such as oranges or lemons, it contains appreciable amounts of vitamins A, B1, B2, niacin (B3), and pantothenic acid (B5), as well as appreciable amounts of phosphorus, calcium, iron, potassium, and sodium (Rodríguez Medina & Valdés-Infante Herrero, 2016).

Various studies have also described the green synthesis of metallic nanoparticles and simple oxide systems using plant extracts, but few have focused on Ag and Au NP or metal oxide nanocomposites obtained through phytogetic routes with guava leaf extracts (Johurul Islam *et al.*, 2023; Nguyen *et al.*, 2023; Patil & Rane, 2020; Santhoshkumar *et al.*, 2014; Sougandhi & Ramanaiah, 2020). To the best of our knowledge, *P. guajava* fruit extract has not been explored as a reducing and stabilizing agent in this specific nanocomposite. The high bioavailability of this biomass, coupled with its great diversity of phytochemicals with reducing properties, makes the guava fruit an ideal candidate for the biogenic synthesis of NPs. Here, we report a silver-functionalized copper oxide and iron oxide nanocomposites biogenic route synthesis using *P. guajava* aqueous fruit extract as a reducing and stabilizing agent. We used X-ray diffraction (XRD), vibrating sample magnetometry (VSM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR) techniques for the structural, magnetic, and morphological characterization of the materials. Finally, we evaluated the bactericidal properties of the materials against strains of *K. pneumoniae*, a Gram-negative microorganism implicated in nosocomial diseases highly resistant to various antimicrobials and antibiotics (Effah *et al.*, 2020). The integration of green synthesis with comprehensive structural and magnetic characterization, together with antibacterial assessment against *K. pneumoniae*, highlights the novelty and application potential of our study, particularly in Colombia, where guava is widely available.

## Experimental details

### Materials

Ripe guava fruit (variety Palmira-ICA1 reported by Otálora *et al.*, 2022) was sourced from the primary wholesale market in Tunja, Boyacá, Colombia; 98% purity iron sulfate heptahydrate  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (CAS No. 7782-63-0) was purchased from Supelco-Ensure; 98% purity copper sulfate pentahydrate  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (CAS No. 7758-99-8) was purchased from Merck; 99.9% purity silver nitrate  $\text{AgNO}_3$  (CAS No. 7761-88-8) was purchased from Loba Chemie, and sodium hydroxide NaOH (CAS No. 1310-73-2) from PanReac AppliChem. All reagents were analytical grade.

### Guava fruit extract preparation

Guava fruit extract was prepared using the modified method reported by Kumar *et al.* (2017). The fruit was thoroughly washed, ground, and filtered on a wide pore mesh; 10 g of the pulp obtained was taken and heated at 50°C in 50 mL of distilled water for 60 min under constant stirring at 1000 rpm and cooled at room temperature. The translucent, pink-colored extract was filtered with Whatman No. 1 filter paper, and then an extract/NaOH solution was prepared by dissolving 25.5 g of guava pulp in 60 mL of distilled water. Once the extract was obtained, 0.5 g of NaOH was added and completely dissolved.

### Preparation of nanocomposites

The samples were obtained using the modified method reported by Bhushan *et al.* (2019). A stoichiometric amount of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was dissolved in 50 mL of distilled water at 50 °C. Then, 50 mL of a  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution was added to reach a  $\text{Cu}^{2+}:\text{Fe}^{2+}$  1:1 molar ratio and, subsequently, 10 mL of the guava extract was added under constant stirring (1000 rpm) for 1 h. The pH was adjusted to 12, drop by drop, through the addition of a 0.2 M NaOH solution. Finally, the sample was aged at 50°C for 24 h to allow the NPS precipitation. The mixture was centrifuged and washed thrice at 2500 rpm for 20 min, then dried at 80 °C for 12 h. This procedure was repeated without the extract to obtain two systems: with extract (FeCu-G) and without it (FeCu).

### **Silver functionalization**

Nanocomposite functionalization was done using the modified method reported by **Kootti et al.** (2013). Initially, 0.7 g of the material was dispersed in 50 mL of 0.05/0.202 (M)  $\text{AgNO}_3$ /Urea solution at room temperature and constant stirring (500 rpm) for 30 min to ensure the adsorption of Ag on the NPs, then, 10 mL of extract/NaOH solution was added to act as a stabilizing agent. After 5 minutes of reaction, 4 mL of guava extract was added as a reducing agent. The mixture obtained was heated to 70°C for 1 h to accelerate the reduction reaction. The functionalized nanocomposite was washed and centrifuged with distilled water at 3500 rpm for 20 min to remove any excess protective agents and alkaline materials, resulting in two samples: FeCuAg5 (without extract) and FeCuAg5-G (with extract). The  $\text{AgNO}_3$ /Urea ratio changed to 0.10/0.404 (M), obtaining the FeCuAg10 and FeCuAg10-G samples.

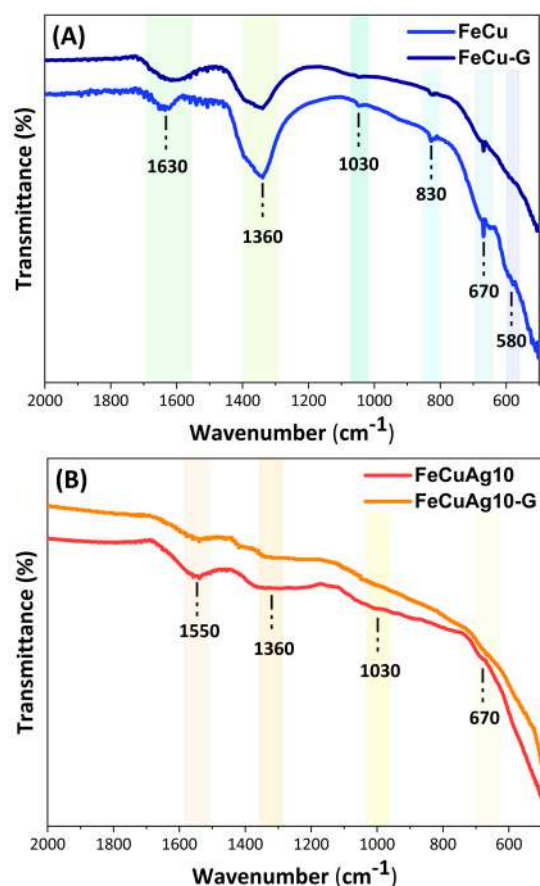
### **Characterization**

The analysis was done in a Nicolet iS50 FTIR equipment using the attenuated total reflectance or ATR technique, recorded with a ZnSe crystal. The infrared spectra were collected in the wavenumber range from 2000  $\text{cm}^{-1}$  to 500  $\text{cm}^{-1}$ . For the crystalline structure analysis by XRD, we used an Empyrean diffractometer, with Cu-K $\alpha$  radiation ( $\lambda=1.5406 \text{ \AA}$ ) between 10° and 90°, a step width of 0.053°, a scanning speed of 2 $\theta$  equals 2° per minute, and a measurement temperature of 293 K. For the TEM study, we used a LEO 440i scanning electron microscope, coating the samples with a thin Au-Pd layer, for the SEM analysis, a CARL ZEISS EVO MA 1 microscope, and to measure the fundamental magnetic properties of the synthesized materials, a Quantum Design Versalab magnetometer. Field-dependent magnetization measurements were taken from -30 to 30 kOe at room temperature (300 K). For temperature-dependent magnetization measurements, we used the Zero Field Cooled-Field Cooled (ZFC-FC) model varying the temperature in the 50 K- 400 K range at an applied field of 50 Oe. We evaluated the inhibition of bacterial growth by the nanocomposites using the microdilution technique in broth. We prepared and diluted 1 mg/mL (1000 ppm) solutions of each synthesized sample in microplates with the bacterial *K. pneumoniae* strain culture broths, starting from 30 ppm. Sodium hypochlorite (NaClO) at 7% was used as a control. The microplates were analyzed at 620 nm using an FC Multiskan TM plate reader.

## **Results and discussion**

### **Structural characterization**

**Figure 1A** shows the infrared spectra corresponding to the FeCu and FeCu-G samples. The characteristic CuO bands were observed at shorter wavenumber, around 580  $\text{cm}^{-1}$ , indicating the vibrational modes of the bond between Cu-O atoms. Additionally, we observed the Cu-O stretching vibration bands around 1360  $\text{cm}^{-1}$  (**Buledi et al.**, 2021; **Sharmila et al.**, 2018). This broadband from 500 to 900  $\text{cm}^{-1}$  and the individual sharp peaks at 830  $\text{cm}^{-1}$  were generally attributed to the metal-oxygen bond, indicating the Fe-O and Cu-O stretching vibration mode (**Jansanthea et al.**, 2024; **Khalil et al.**, 2017; **Nope et al.**, 2025). The peak around 670  $\text{cm}^{-1}$  responds to the Cu(I)-O bond (**Al-Senani et al.**, 2022; **Castañeda-Mendoza et al.**, 2025), their drastic decrease indicating a compositional change involving  $\text{Cu}_2\text{O}$ . A subsequent XRD analysis confirmed this transformation. The band around 1630  $\text{cm}^{-1}$  was associated with the vibrational modes of the O-H bond bending due to the superficial water adsorption (**Amin et al.**, 2016; **Apte et al.**, 2007). In **Figure 1B**, the new band around 1550  $\text{cm}^{-1}$  is usually attributed to the N-H bond vibrational modes and is common in these biogenic routes (**Sivaraj et al.**, 2014). Furthermore, the use of urea in the functionalization process can explain the presence of this functional group. In general terms, the detection of fine spectral peaks between 577  $\text{cm}^{-1}$  and 1030  $\text{cm}^{-1}$ , with differentiated absorption bands for copper and iron oxides, corroborated the presence of these oxides in the samples, where the differences between the IR spectra of the functionalized and non-functionalized samples were attributed to the nanocomposites coating by Ag NPs.



**Figure 1.** FTIR spectra of FeCu and FeCu-G (A) and FeCuAg10 and FeCuAg10-G samples (B)

Other authors have documented the variations between coated materials' IR spectra and those of uncoated or non-functionalized materials (Chitradevi *et al.*, 2019; Kumar *et al.*, 2019; Singh *et al.*, 2022), which can be attributed to the surface interaction of phytochemicals present in the extract. Literature reports demonstrate that polyphenols, flavonoids, proteins, and organic acids, including O–H, N–H, and C=O groups, adsorb onto metallic or oxide surfaces during biogenic synthesis, leading to shifts or intensity changes in FTIR peaks (Kumar *et al.*, 2016). Furthermore, any variation in particle size or morphology induced by the extract may enhance surface area effects, amplifying the superficial functional groups as observed in O–H bound spectral changes.

On the other hand, the XRD pattern of the non-functionalized NPs (Figure 2) shows six peaks in the whole spectrum, with  $2\theta$  values of 35.62°, 36.42°, 38.80°, 42.30°, 61.44°, and 73.52° for the FeCu and FeCu-G samples. Using the X'pert high score Plus software and the Crystallography Open Database (COD), these signals were assigned to three crystalline phases: a first phase of iron (III) oxide (Fe<sub>2</sub>O<sub>3</sub>) (ICDD 01-073-2234), presenting a rhombohedral structure with space group R3-C (167), lattice parameters  $a = b = 5.0342$  Å and  $c = 13.7483$  Å, with angles  $\alpha = \beta = 90^\circ$  and  $\gamma = 120^\circ$ , a relative abundance of 95% in FeCu and 93% in FeCu-G; a second phase of copper(I) oxide or (Cu<sub>2</sub>O) (ICDD 01-078-2076) with cubic structure and space group Pn-3m (224), lattice parameters  $a = b = c = 4.2670$  Å with angles  $\alpha = \beta = \gamma = 90^\circ$ , and a relative abundance of 3% in FeCu and 6% in FeCu-G, with the use of guava fruit extract increasing the formation of Cu<sub>2</sub>O, and a last phase of copper(II) oxide (CuO) (ICDD 01-089-2529) with a monoclinic structure and space group C2/c (15), lattice parameters  $a = 4.6853$  Å,  $b = 3.4257$  Å,  $c = 5.1303$  Å of angles  $\alpha = \gamma = 90^\circ$ ,  $\beta = 99.549^\circ$ , relative abundance of 2% in FeCu and 1% in FeCu-G, a

reduction due to the reactions generated by the multicomponent matrix of guava extract, transforming and stabilizing the  $\text{Cu}^{2+}$  ions into  $\text{Cu}^{1+}$ . These results agree with other reports (Mohamed *et al.*, 2021; Parvathiraja & Shailajha, 2021).

The XRD spectra of silver functionalized nanocomposites (Figure 3) revealed peaks of both the nanocomposite support and the immobilized Ag NPs; the signals corresponded to an Ag phase with  $2\theta$  values of  $38.18^\circ$ ,  $44.38^\circ$ ,  $64.52^\circ$ , and  $77.50^\circ$  matching crystallographic planes (111), (200), (220), and (311), respectively, and exhibiting a cubic structure with space group  $Fm-3m$  (225), lattice parameters  $a = b = c = 4.0773 \text{ \AA}$ , and angles  $\alpha = \beta = \gamma = 90^\circ$ . The peaks assigned to the NPs showed lower intensity compared to their non-functionalized counterparts, which has been attributed to the presence of Ag-NPs on the

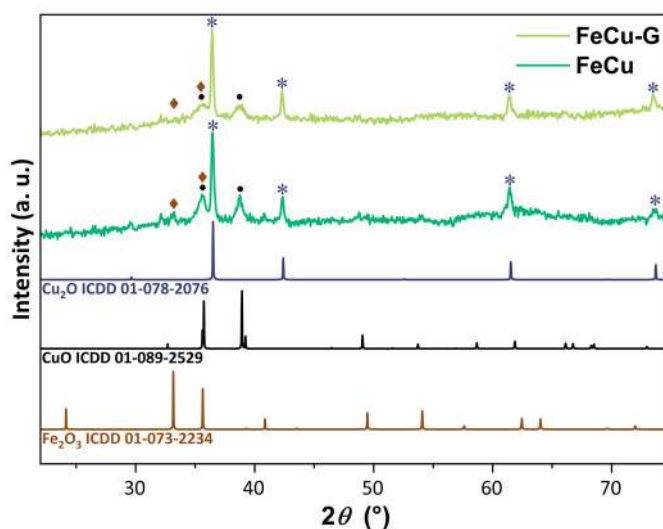


Figure 2. XRD patterns of the unfunctionalized FeCu and FeCu-G samples. The peaks assigned to the detected crystalline phases are indicated with the symbols (\*)  $\text{Cu}_2\text{O}$ , (◇)  $\text{Fe}_2\text{O}_3$ , and (•)  $\text{CuO}$

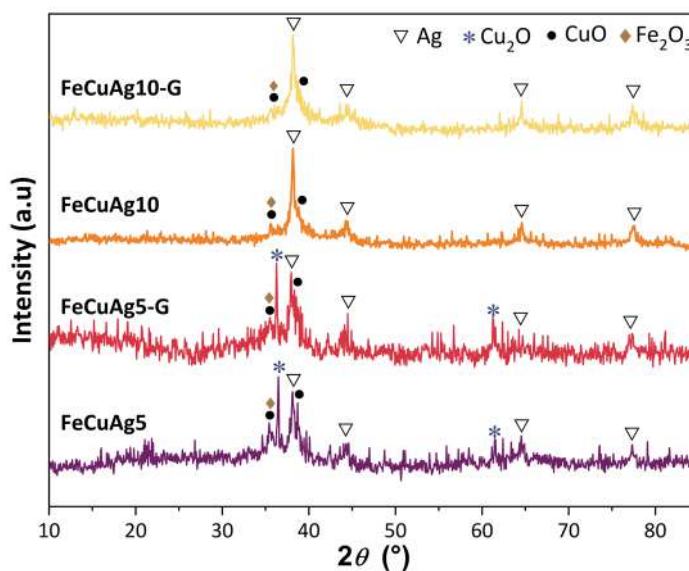


Figure 3. XRD of functionalized samples. The peaks assigned to the Ag crystalline phases are indicated with the symbol (▽). The peaks observed in the diffractograms indicate the crystalline nature of the synthesized solids

surface of the nanocomposites (Kanwal *et al.*, 2019), where the absence of specific peaks in the diffractograms of the functionalized samples may respond to the higher concentration of Ag-NPs.

The reducing and stabilizing capacity of the guava extract can be attributed directly to its phytochemical composition. Previous studies have demonstrated that *P. guajava* is rich in bioactive metabolites such as flavonoids, polyphenols, tannins, carotenoids, and ascorbic acid, all of which exhibit well-documented redox activity and surface-capping ability in green synthesis routes (Nguyen *et al.*, 2023; Otálora *et al.*, 2022, 2024). These compounds may actively contribute to nanoparticle nucleation, growth regulation, and surface stabilization, while also enhancing the antibacterial functionality of the resulting nanocomposites. Although we did not evaluate here the influence of the guava variety, ripening stage, or agroecological conditions on the composition of the extract, these factors are known to affect the concentration and stability of bioactive molecules (Ibrahim *et al.*, 2019; Nguyen *et al.*, 2023).

These structural modifications are not only indicative of the successful silver functionalization and the interaction of phytochemicals with the oxide-based composite surface, but they also have direct implications for the antibacterial behavior of the nanocomposites. Previous studies have shown that silver-functionalized copper and iron oxides exhibit enhanced antibacterial performance due to a synergistic mechanism where structural defects, exposed surface functional groups (–OH, –NH), and Ag-related surface reactivity facilitate reactive oxygen species (ROS) generation and membrane disruption in Gram-negative bacteria (Antonoglou *et al.*, 2019; Pareek *et al.*, 2021; Parvathiraja & Shailajha, 2021).

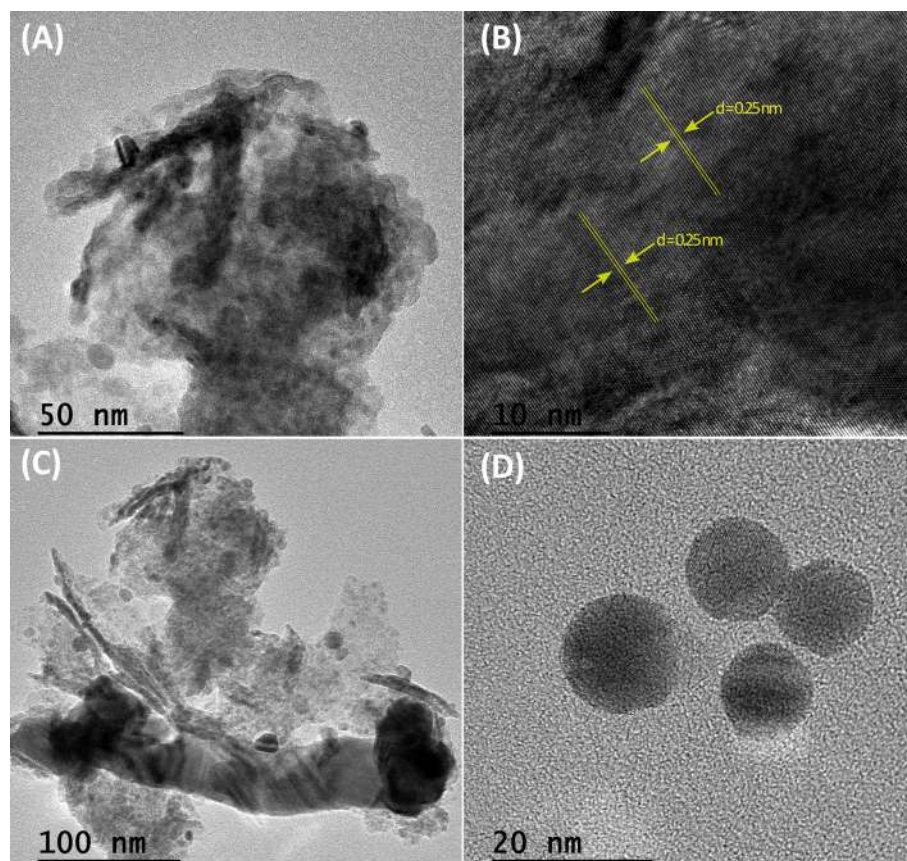
### ***Structural morphological analyses***

The TEM and HR-TEM images of the FeCuAg5-G nanocomposite are shown in **Figure 4A-D**. Agglomerated nanosized crystals can be observed in these images (A, C), a common characteristic of polycrystalline materials (Abd El-Sadek *et al.*, 2019). The morphology of the compounds analyzed is circular and heterogeneous, and the contrasts observed in the images arise due to the different orientations of the crystals in relation to the electron beam (Yalcin *et al.*, 2014). The particles observed have diameters ranging between 7.10 nm and 19.40 nm, with an average diameter of  $7.69 \pm 0.57$  nm. The lattice lines correspond to the interplanar distances in **Figure 4B**, which were calculated using HR-TEM and measured on a 10 nm scale. The highly crystalline structure with an interplanar distance of  $d = 0.25$  nm on the plane (002), consistent with the monoclinic phase of CuO, matched the XRD data and reports (Pallavolu *et al.*, 2023). On the other hand, it was possible to observe the silver nanoparticles with a defined spherical shape (**Figure 4D**).

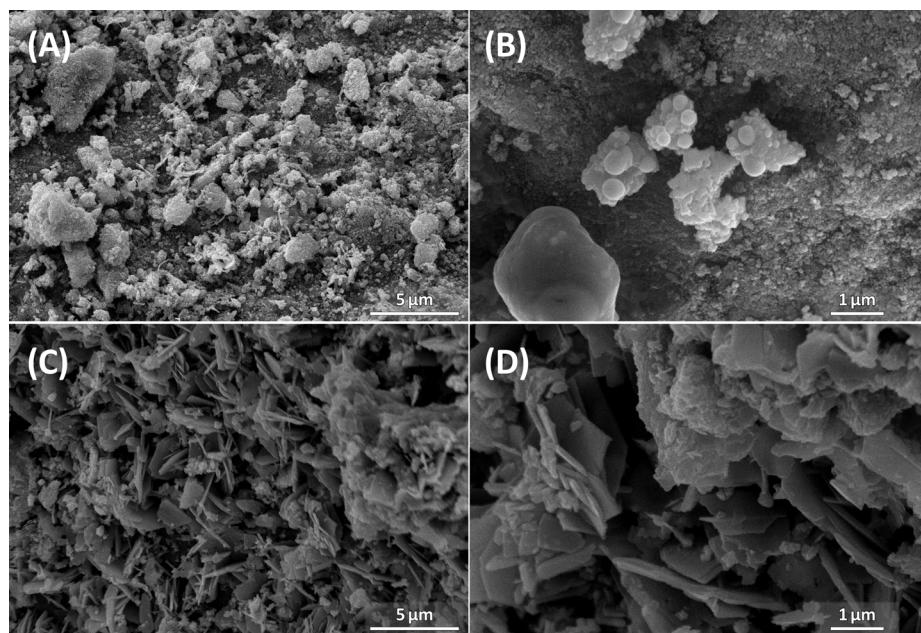
Sample FeCuAg10 SEM image (**Figure 5 A-B**) shows aggregates without a specific shape, with an average particle size of  $103.7 \pm 7.3$  nm. In contrast, sample FeCuAg10-G (**Figure 5 C-D**) had nanoflakes structures with defined edges and smooth surfaces ( $79.54 \pm 4.41$  nm). This flake morphology is attributed to the presence of copper oxide CuO (Siddiqui *et al.*, 2016), so the elongated CuO distribution corresponds to the preferential orientation of this oxide along the crystallographic plane (010) (Hwa *et al.*, 2019; Pallavolu *et al.*, 2023). The spherical particles in both samples are due to Ag NPs, while the other heterogeneous phase is attributed to the Fe<sub>2</sub>O<sub>3</sub> phase.

### ***Magnetic characterization***

**Figure 6** shows the magnetization vs field isotherm at 300 K for FeCuAg10 and FeCuAg10-G samples. The coercivity field ( $H_c$ ) and remanent magnetization ( $M_r$ ) (Gaona *et al.*, 2023; Saavedra-Gaona *et al.*, 2024) were determined for FeCuAg10, with  $M_r = 0.0011$  emu/g and  $H_c = 11$  Oe, and for FeCuAg10-G, with  $M_r = 0.00034$  emu/g and  $H_c = 5$  Oe. The hysteresis loops depict weak ferromagnetic behavior with a predominant paramagnetic contribution. This behavior is attributed to particle size reductions, oxygen vacancies, and spin ordering (Atchaya & Meena Devi, 2024). Additionally, nanostructure anisotropy, oxygen vacancies, structural disorder, defects, surface spins, and magnetic exchange coupling contribute to these properties (Al-Saeedi *et al.*, 2021; Atchaya & Meena Devi, 2024; Batsaikhan *et al.*, 2020).



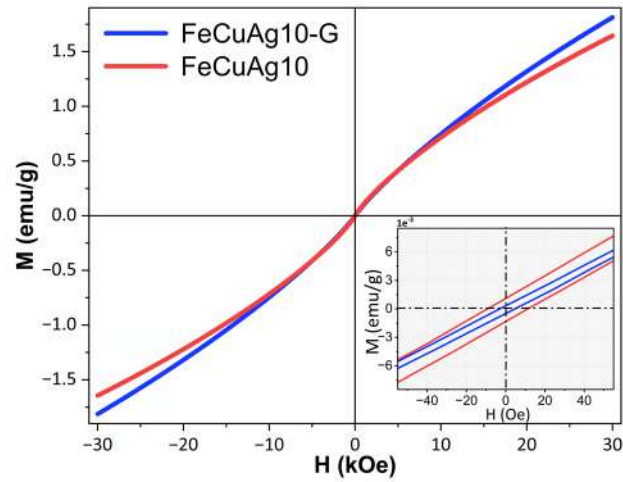
**Figure 4.** TEM and HR-TEM images of the FeCuAg5-G sample. The TEM images display polycrystalline agglomerates with spherical shape, whereas the HR-TEM analysis reveals a d-distance value of  $\sim 0.25$  nm



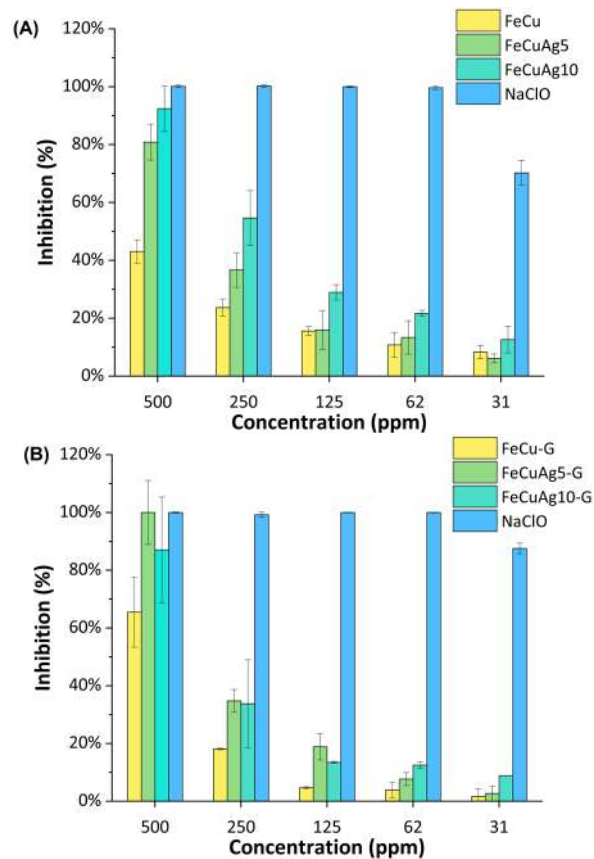
**Figure 5.** SEM images for the FeCuAg10 (A-B) and FeCuAg10-G (C-D) samples

**Bactericidal activity**

The microdilution tests evaluated the bactericidal activity of the synthesized nanocomposites against the Gram-negative *K. pneumoniae* strain. Bacterial growth inhibition percentages for different nanocomposite concentrations are presented in **Figure 7**; the maximum bacterial growth inhibitory capacity of functionalized and non-functionalized



**Figure 6.** Magnetic isotherms at 300 K of FeCuAg10 and FeCuAg10-G samples



**Figure 7.** Bactericidal response against *Klebsiella pneumoniae*

nanocomposites occurred at 500 ppm, in contrast with the NaClO positive target 7% concentration (70,000 ppm). The bactericidal potential of the nanocomposites against the target was evident, which is even more relevant when considering the high resistance prevalence rates of this bacterium against most commonly used antimicrobial drugs, including cefepime, aztreonam, and cefotaxime, with 72.6%, 73.3%, and 79.2%, respectively (Effah *et al.*, 2020).

Although the microdilution tests showed an inhibitory effect on bacterial growth for all nanocomposites, it was higher in the functionalized samples, probably explained by the fact that Fe<sub>2</sub>O<sub>3</sub> NPs, for example, tend to exhibit greater bactericidal activity against Gram-positive bacteria than against Gram-negative bacteria, which is attributed to the fact that these NPs are highly stable under normal environmental conditions, thus generating fewer metal ions releases that exert bactericidal activity (Pallela *et al.*, 2019). On the other hand, Parek *et al.* (2021) explained how bactericidal response improvement in functionalized solids is due to Ag NPs generating intra- and extracellular ROS in *K. pneumoniae*, which, consequently, produces oxidative stress leading to cellular demise. Also, Ag NPs can damage bacterial DNA and inhibit microbial growth by releasing Ag<sup>+</sup> ions, which interact with sulfur compounds, leading to cell death. (Shahriary *et al.*, 2018).

## Conclusions

The structural characterization confirmed the synthesis of copper oxide and iron oxide functionalized with silver NPs. The use of guava fruit extract not only modified the phase composition, increasing the presence of Cu<sub>2</sub>O, but also promoted the formation of nanoflake-like morphologies, highlighting its role as a sustainable capping and reducing agent. Magnetic evaluation revealed predominantly paramagnetic behavior with weak ferromagnetic contributions. Notably, the incorporation of the extract led to reduced saturation magnetization and coercivity, evidencing the influence of phytochemicals on the magnetic response. Besides, the biogenic route enhanced the antibacterial activity of the nanocomposites, as functionalized samples synthesized with guava extract exhibited stronger inhibition against *K. pneumoniae*. These findings demonstrate that green synthesis using guava extract provides a viable pathway for tailoring structural, magnetic, and antibacterial properties, underscoring its potential for biomedical and environmental applications.

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## Author contributions

**CAPV, RJRO:** supervision. **MCM, GLMN:** material preparation. **CCRF, CLSS, IMSG:** characterization. **MCM, GLMN, RJRO, IMSG, DLLP:** methodology and formal analysis. **MCM, GLMN:** drafting of the initial manuscript. All authors conducted conceptualization and investigation, and reviewed and approved the final manuscript.

## Conflicts of interest

The authors declare there are no conflicts of interest regarding the publication of this article. They also declare that they have no financial or personal relationships that could influence their work or affect the interpretation of the results presented in this article

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