Research Article


Green synthesis of ZnO nanoparticles using the mangosteen (Garcinia mangostana L.) leaf extract: Comparative preliminary in vitro antibacterial study

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Abstract: In the realm of public health, the rising threat caused by bacteria resistant to many drugs is a critical concern. In this work, we used the aqueous extract of mangosteen leaves to create zinc oxide (ZnO) nanoparticles (NPs) in an environmentally friendly manner. Through various analytical methods, we thoroughly characterized these biogenic ZnO NPs, including UV–visible, Fourier transform infrared, X-ray photoelectron spectroscopy, Raman spectroscopy, X-ray powder diffraction, field emission-scanning electron microscopy with energy dispersive X-ray and high resolution-transmission electron microscopy. ZnO NPs showed distinctive properties among different characterization techniques, including a small energy bandgap of 2.80 eV, a porous, a minimum crystalline size of 16.99 nm, an average particle size of 14.21 nm, and a spherical nanostructure. Additionally, we performed preliminary antibacterial experiments to assess ZnO NPs, copper oxide (CuO) NPs, and ZnO–CuO nanocomposites for antibacterial activity. Interestingly, ZnO NPs showed significant potential in suppressing the growth of Staphylococcus aureus ATCC BAA-1026, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922, and Klebsiella pneumoniae ATCC 13883, with decreasing order of minimum inhibitory concentrations: S. aureus = B. subtilis (15.63 μg·mL⁻¹) > E. coli (62.50 μg·mL⁻¹) > K. pneumoniae (125.00 μg·mL⁻¹). These results highlight the potential of biogenic NPs, particularly ZnO NPs, as effective agents against multi-drug-resistant bacteria.

Keywords: green synthesis, Garcinia mangostana L., antibacterial activity, nanoparticles, public health

1 Introduction

The advent of antibiotics in the mid-twentieth century marked a significant milestone in the field of medicine. However, the widespread misuse and overuse of antibiotics have given rise to the emergence of multi-drug-resistant
bacteria, rendering nearly all existing antibiotics ineffective [1–3]. Despite ongoing efforts to introduce new antibiotics to the market, production rates have proven inadequate to counter the rapid emergence of multi-drug-resistant bacterial strains [1]. Consequently, the proliferation of pathogenic bacterial outbreaks, driven by resistance to conventional antibiotics, has emerged as a pressing global public health crisis in the twenty-first century [4]. As affirmed by the World Health Organization (WHO), bacterial infections are responsible for millions of annual deaths worldwide [2,5]. Consequently, researchers have turned their attention toward nanomaterials as a promising alternative solution [6]. Nanoparticles (NPs) have garnered particular interest due to their distinctive mechanisms of action in combating bacteria. They can directly interact with bacterial cell walls, obviating the need for cellular penetration [7], and can disrupt biochemical pathways by damaging organelles, ultimately leading to bacterial cell death [2].

Zinc oxide (ZnO) is a n-type semiconductor that ranges in band gap energy from 3.30 to 3.37 eV with a substantial exciton binding energy of 60 meV [8–10]. It also has strong bonding characteristics [11], near-ultraviolet emission [12], and remarkable photo- and thermal stability at room temperature [13]. The piezoelectricity of this material is extensively used in attenuators and sensors [12]. Moreover, the US Food and Drug Administration has classified ZnO as a “generally recognized as safe” chemical since it is non-toxic [14–16]. As a result, ZnO-containing medications are safe to use without a coating. ZnO can also be utilized in sunscreen creams, fabrics, and coatings due to its capacity to filter UV radiation and suppress antibacterial characteristics [12]. Additionally, ZnO is being used extensively in biological, catalytic, sensor, energy storage, photocatalyst, and optoelectronic devices [10,12,17]. On the other hand, compared to other noble metals, copper oxide (CuO), a p-type semiconductor with an energy bandgap ranging from 1.02 to 2.0 eV [9,18,19], provides more favourable economic advantages [20]. It is easily compatible with polymers due to its stability [21]. Superior thermal conductivity, optical characteristics, electron correlation effects, spin dynamics, magnetic phases, antioxidant capacity, and antibacterial properties are all exhibited by CuO NPs [21–24]. CuO NPs are therefore used in magneto-resistant materials, biosensors, antibacterial agents, supercapacitors, magnetic storage media, and field emission devices [22,24,25]. Recent attention has turned toward the design of nanocomposites (NCs), including ZnO–CuO, which promise enhanced performance in various applications [9]. ZnO–CuO NCs are one of the most studied p–n type heterojunction semiconductors because copper can easily overlap d-electrons with the valence bond of ZnO [26,27]. CuO is, therefore, the best metal oxide to be used in the synthesis of NCs with ZnO because it effectively separates and transfers photo-excited electrons from the upper conduction band to the lower one. ZnO–CuO NCs function very well as photocatalysts due to their huge surface area and simple production techniques [28,29]. However, in vitro preliminary antibacterial activity involving ZnO–CuO NCs remains sparsely reported to date.

In contrast to conventional synthesis methods like thermal decomposition, coprecipitation, and sol–gel processes, the green synthesis of nanomaterials offers advantages such as reduced cost, lower energy consumption, simplicity, and environmental friendliness. In general, microbes, plant extracts, viruses, fungi, yeasts, microalgae, and macroalgae can all be used to carry out the green synthesis of NPs [10,30,31]. Different from biological entities, plant extracts are more dependable, straightforward, and environmentally friendly than other biological entities when it comes to the green synthesis of NPs [32–34]. Plant-mediated green synthesis also presents several benefits over other biological materials for the synthesis of NPs. These include the ability for one-spot synthesis of NPs, as well as robustness, eco-friendliness, natural capping and reducing agents, ease of availability, safety, cost-effectiveness, suitability for large-scale synthesis, and not requiring cell cultures [19,34,35].

Compared to conventional methods applied, large-scale production of green-synthesized NPs from lab scale to pilot-plant level is still in the early stage [31]. Therefore, mangosteen, which has high nutrient value, was selected as a bio-reducing agent in synthesizing NPs in this study. *Garcinia mangostana* L., commonly known as mangosteen and belonging to the Clusiaceae family, is rich in phytochemicals such as xanthones, flavonoids, and terpenes. These compounds possess antibacterial, anti-tumour, anti-diabetic, immunomodulatory, anti-allergic, and hepatoprotective properties [36–39]. Mangosteen is a seasonal tropical fruit found in regions such as Malaysia, Thailand, Indonesia, and others. Given its abundant phytochemical content, mangosteen exhibits the potential to serve as a stabilizing agent for colloidal nanomaterials [40–43].

Both CuO NPs and ZnO-CuO NCs were synthesized through a green approach using mangosteen leaf aqueous extract as natural capping and reducing agents and detailed physicochemical properties of these materials have been reported in previously published studies [44,45]. This study focuses on the green synthesis of ZnO NPs using mangosteen leaf aqueous extract. We explore their optical, structural, and morphological properties, along with conducting and comparing their *in vitro* preliminary antibacterial assessments with CuO NPs and ZnO–CuO NCs.
2 Materials and methods

The mangosteen leaves were collected from Kampar, Malaysia. Zinc nitrate hexahydrate, Zn(NO$_3$)$_2$·6H$_2$O, and nutrient broth (NB) were purchased from HiMedia Laboratories Pvt. Ltd. (Nashik, India). Meanwhile, copper(n) nitrate trihydrate [Cu(NO$_3$)$_2$·3H$_2$O] was purchased from HmbG (Hamburg, Germany). The chemicals were used without further purification. The glassware was washed with deionized water and dried in an oven before use. Both Gram-positive (B. subtilis; ATCC 6633), Gram-negative (E. coli; ATCC 25922 and K. pneumoniae; ATCC 13883) bacteria were obtained from Faculty of Sciences, Universiti Tunku Abdul Rahman (UTAR), Malaysia.

2.1 Preparation of mangosteen leaf aqueous extract

The process of preparing the aqueous extract from mangosteen leaves was modified from the study of Chan et al. [44]. To get rid of the dust from their surface, the mangosteen leaves were carefully rinsed with tap water. The cleaned leaves were dried for a total of 48 h in an oven and an additional 8 h in a vacuum oven. The dried leaves were then processed in a grinder to form a fine powder. Subsequently, 4.0 g of finely powdered mangosteen leaves was added to 100 mL of deionized water, heated, and stirred at 70–80°C for 20 min to produce 0.04 g mL$^{-1}$ of the leaf aqueous extract. The leaf aqueous extract was vacuum-filtered, and a reddish-brown filtrate was obtained for the synthesis of ZnO NPs after cooling to room temperature.

2.2 Synthesis of ZnO NPs

Similarly, the green synthesis of ZnO NPs was adapted from the study of Chan et al. [44]. The synthesis of ZnO NPs was performed by using mangosteen leaf aqueous extract. The reaction parameters, which included the mangosteen leaf aqueous extract concentration and calcination temperature, were optimized.

2.2.1 Leaf aqueous extract optimization

A 50 mL of the prepared mangosteen leaf aqueous extract (0.02, 0.03, and 0.04 g mL$^{-1}$) was heated and stirred at 70–80°C. During heating, 4.0 g of Zn(NO$_3$)$_2$·6H$_2$O was added to the leaf aqueous extract, and a light brown solution was formed. Meanwhile, heating and constant stirring were continued at 70–80°C until the formation of a dark brown paste, which was then cooled to room temperature before it was transferred to a ceramic crucible and calcined at 400°C for 2 h in a Muffle furnace. Finally, a fine white ZnO powder was obtained.

2.2.2 Calcination temperature optimization

After the selection of optimized mangosteen leaf aqueous extract concentration at 0.04 g mL$^{-1}$, the synthesis of ZnO NPs was repeated by using 4.0 g of Zn(NO$_3$)$_2$·6H$_2$O. The cooled dark-brown paste was calcined at 300°C, 400°C, and 500°C for 2 h in a Muffle furnace.

2.3 Synthesis of CuO NPs and ZnO–CuO NCs

ZnO NPs and CuO-ZnO NCs were synthesized using the above-mentioned method. About 0.05 g mL$^{-1}$ of mangosteen leaf extract and 2.0 g of Cu(NO$_3$)$_2$·3H$_2$O were calcined at 500°C for 2 h to obtain a black powder of CuO NPs [44]. Meanwhile, 0.05 g mL$^{-1}$ mangosteen leaf extract with 4.0 g of Zn(NO$_3$)$_2$·6H$_2$O and 2.0 g of Cu(NO$_3$)$_2$·3H$_2$O were calcined at 500°C for 2 h to obtain dark-brown ZnO–CuO NC powder [45].

2.4 Characterization

The structural, morphological, and optical properties of the green-synthesized ZnO NPs were characterized by using various analytical tools. The absorption spectra were recorded using a UV-visible (UV-Vis) spectrophotometer (Thermo Scientific GENESYS 10S). The Fourier transform infrared (FT-IR) spectroscopy was performed in the range of 4,000–400 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ using KBr pellets in a Perkin Elmer RXI spectrophotometer. X-ray photoelectron spectroscopy (XPS) was performed to determine the oxidation state by using a Kratos- Shimadzu Axis Ultra DLD model. Also, a micro-Raman spectrophotometer (Thermo Scientific DXR2xi model) was used to measure the Raman spectra. X-ray powder diffraction (XRD) patterns were taken in the reflection mode with Cu Ka ($\lambda = 1.5406 \text{ Å}$) radiation in the 20 range from 10 to 80° by using a Shimadzu XRD 6000 X-ray diffractometer by continuous scanning, operated at 40 kV/30 mA and 0.02 min$^{-1}$. The morphological, microstructural, and elemental compositions of the synthesized samples were determined using a field emission-scanning electron microscope (FE-SEM, JEOL JSM-6710F, Japan) with an energy dispersive X-ray analyser (EDX, X-max, 150 Oxford Instruments) and high resolution-transmission electron microscope (HR-TEM, TECNAI G2 20 S-TWIN, FEI) at 200 kV.
2.5 In vitro antibacterial screening

The broth dilution assay was used and adopted by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) discussion document in 2003 [46]. The bacteria colonies were transferred to sterilized NB and incubated overnight at 35–37°C. The culture was adjusted to $1–2 \times 10^8$ CFU·mL$^{-1}$ with sterilized NB photometrically (absorbance reading in the range of 0.08–0.10 at 600 nm). The bacterial suspension was further diluted in sterilized NB to obtain $1–2 \times 10^5$ CFU·mL$^{-1}$. Two-fold dilutions of green-synthesized ZnO NPs, CuO NPs, and ZnO–CuO NCs suspensions (1,000.00, 500.00, 250.00, 125.00, 62.50, 31.25 and 15.63 μg·mL$^{-1}$) and antibiotic (ampicillin salt solution, 100.00, 50.00, 25.00, 12.50, 6.25, 3.13 and 1.56 μg·mL$^{-1}$) in sterilized NB were dispensed in sterilized tubes and inoculated with $1–2 \times 10^5$ CFU·mL$^{-1}$ bacterial suspension. After incubation overnight at 35–37°C, the visible bacterial growth in tubes was examined by turbidity with a minimum inhibitory concentration (MIC). The study was carried out in triplicate.

2.6 Statistical analysis

The MIC values of the synthesized ZnO NPs, CuO NPs, and ZnO–CuO NCs are presented as mean ± standard deviation (SD). Two-way ANOVA and Tukey’s test were performed to compare the MIC values of the different nanomaterials synthesized from the mangosteen leaf aqueous extract and tested with different bacteria, using Microsoft Excel 2013 by setting a $p$-value < 0.05 as a significant criterion.

3 Results and discussion

3.1 Characterization of ZnO NPs

The physicochemical properties of ZnO NPs are tabulated in Table 1, and the comparison with other studies are summarized in Table 2.

3.1.1 Bond features of organic and inorganic material analysis

FT-IR spectroscopy was used to examine the bond characteristics of the organic and inorganic components in the green-synthesized ZnO NPs, as shown in Figure 1. $\nu$(O–H) and $\nu$(C═O) correspond to 3,431–3,436 and 1,628–1,634 cm$^{-1}$. 

<table>
<thead>
<tr>
<th>Mangosteen leaf aqueous extract concentration (g·mL$^{-1}$)</th>
<th>Energy bandgap (eV)</th>
<th>Crystalline size (nm)</th>
<th>Dislocation density ($\times 10^{14}$ cm$^{-1}$)</th>
<th>Micron strain ($\times 10^{-4}$)</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>400</td>
<td>3.28</td>
<td>27.63</td>
<td>13.10</td>
<td>Irregular nanostructure</td>
</tr>
<tr>
<td>0.03</td>
<td>400</td>
<td>3.27</td>
<td>21.50</td>
<td>16.71</td>
<td>Quasi-spherical nanostructure</td>
</tr>
<tr>
<td>0.04</td>
<td>300</td>
<td>3.37</td>
<td>35.49</td>
<td>21.3</td>
<td>Spherical nanostructure</td>
</tr>
<tr>
<td>0.04</td>
<td>500</td>
<td>2.89</td>
<td>34.63</td>
<td>2.08</td>
<td>Spherical nanostructure</td>
</tr>
</tbody>
</table>
respectively. Moreover, 1,384 and 1,110–1,124 cm\(^{-1}\) bands were assigned to \(\nu(C-C\text{ aromatic})\) and \(\nu(C-O)\). A sharp and intense band located at 456–510 cm\(^{-1}\) appeared in green-synthesized ZnO NPs, indicating the presence of \(\nu(Zn-O)\). As shown in Figure 1(a) and (b), the ZnO NPs band’s intensity located at 1,628–1,634 cm\(^{-1}\) increased when a higher concentration of the mangosteen leaf aqueous extract was utilized, whereas it decreased between 1,116 and 1,124 cm\(^{-1}\). On the other hand, the band’s intensity at 1,384 cm\(^{-1}\) declined; meanwhile, the band’s intensity increased at 1,110–1,124 cm\(^{-1}\) intensity at elevated calcination temperature.

### 3.1.2 Optical and energy bandgap analysis

Figure 2 displays the UV-Vis spectra of Zn(NO\(_3\)\(_2\))\(_6\)H\(_2\)O solution, mangosteen leaf aqueous extract, ZnO NPs synthesized using different concentrations of mangosteen leaf aqueous extract and calcination temperatures. The aqueous extract of mangosteen leaves has a wide absorption peak at 479 nm, whereas the Zn(NO\(_3\)\(_2\))\(_6\)H\(_2\)O absorption peak was measured at 305 nm. Meanwhile, the absorbance maxima of the green-synthesized ZnO NPs were detected between 367 and 373 nm, which were shifted to a higher wavelength compared to the Zn(NO\(_3\)\(_2\))\(_6\)H\(_2\)O absorption peak, which was located at 305 nm. The Tauc plot was used to determine the ZnO NPs’ energy bandgap (Figure 3). The energy bandgap of ZnO NPs was calculated (in eV) by plotting \((\alpha h\nu)^2\) against \(h\nu\), where \(h\) is the Planck’s constant \((6.626 \times 10^{-34} \text{ Js})\) and \(\alpha\) is the absorption coefficient. The concentration of the leaf aqueous extract and the calcination temperature both altered the ZnO NPs’ energy bandgap. When a higher concentration of mangosteen leaf aqueous extract was used, the ZnO NPs’ energy bandgap slightly dropped from 3.28 to 3.27 eV. Similarly, the use of a higher calcination temperature resulted in a significant drop in ZnO NPs from 3.37 to 2.80 eV.

### 3.1.3 Crystallinity analysis

Figure 4 shows the XRD patterns of mangosteen leaf aqueous extract-mediated ZnO NPs using different leaf aqueous extract concentrations and calcination temperatures. All the green-synthesized ZnO NPs were in good agreement with ICDD 01-079-9878, with 2\(\theta\) values of 31.66, 34.40, 36.20, 47.58, 56.52, 62.84, 67.90, and 69.28\(^o\). The most well-defined, higher intensity and narrower diffraction peaks were observed in ZnO NPs by using a concentration of lower mangosteen leaf aqueous extract concentrations and calcination temperatures.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Plant extract concentration (g·mL(^{-1}))</th>
<th>Calcination temperature (°C)</th>
<th>Mean particle size (nm)</th>
<th>Energy bandgap (eV)</th>
<th>Calculated morphology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crotalaria verrucosa</em> (leaf)</td>
<td>0.001</td>
<td>400</td>
<td>27.00</td>
<td>3.87</td>
<td>Hexagonal nanostructure</td>
<td>[14]</td>
</tr>
<tr>
<td><em>Cassia fistula</em> (leaf)</td>
<td>0.01</td>
<td>300</td>
<td>13.62</td>
<td>3.83</td>
<td>Nearly spherical nanostructure</td>
<td>[47]</td>
</tr>
<tr>
<td><em>Melia azedarach</em> (leaf)</td>
<td>0.01</td>
<td>350–400</td>
<td>3.24</td>
<td>3.21</td>
<td>Spherical nanostructure</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Cinnamomum tamala</em> (leaf)</td>
<td>0.02</td>
<td>500</td>
<td>19.78</td>
<td>2.80</td>
<td>Spherical nanostructure</td>
<td>Current study</td>
</tr>
<tr>
<td><em>Lippia adoensis</em> Koseret (leaf)</td>
<td>0.05</td>
<td>400</td>
<td>14.21</td>
<td>3.21</td>
<td>Spherical nanostructure</td>
<td>[49]</td>
</tr>
<tr>
<td><em>G. mangostana</em> L. (leaf)</td>
<td>0.04</td>
<td>500</td>
<td>14.21</td>
<td>2.80</td>
<td>Spherical nanostructure</td>
<td>Current study</td>
</tr>
</tbody>
</table>

1 By using dynamic light scattering technique.  
2 1:1 volume ratio of the plant extract and zinc precursor.  
3 The best physicochemical properties with optimum conditions were chosen.
extract, which was calcined at higher temperatures. From the XRD spectra, the intensities of the (1 0 0), (0 0 2), and (1 0 1) peaks were stronger than other peaks. The ZnO NPs were in a hexagonal-wurtzite crystal system with lattice parameters $a = 3.2490 - 3.2648$ Å and $c = 5.1876 - 5.2194$ Å in the $P6_3mc$ space group.

The crystalline size, dislocation density, and microstrain of ZnO NPs were calculated by using Debye–Scherrer’s formula (Eq. (1)) and Williamson and Smallman’s formula (Eqs (2) and (3)), respectively.

$$D = \frac{0.94 \lambda}{\beta \cos \theta}$$  \hspace{1cm} (1)
$$\delta = \frac{1}{D^2}$$  \hspace{1cm} (2)
$$\varepsilon = \frac{\beta \cos \theta}{4}$$  \hspace{1cm} (3)

where $D$ is the crystalline size of NPs, $\lambda$ is the X-ray wavelength, $\beta$ is the full width half-maximum of the peak, $\theta$ is the Bragg angle, $\delta$ is the dislocation density of NPs, and $\varepsilon$ is the microstrain of NPs. Compared to the calcination temperature, the ZnO NPs’ crystallinity was significantly affected by the concentration of the mangosteen leaf aqueous extract. The crystalline size of ZnO NPs was significantly decreased from 27.63 to 16.79 nm with increasing mangosteen leaf aqueous extract concentration. In contrast, the ZnO NPs’ crystalline size slightly increased from 16.71 to 16.99 nm at an elevated calcination temperature. On the other hand, utilizing the concentrated mangosteen leaf aqueous extract and lowering the calcination temperature resulted in an increase in the dislocation density of ZnO NPs from $13.10 \times 10^{-14}$ to $35.49 \times 10^{-14}$ cm$^{-1}$ and $34.63 \times 10^{-14}$ to $35.80 \times 10^{-14}$ cm$^{-1}$, respectively. Similarly, employing a low-concentration of mangosteen leaf aqueous extract (decreased from $2.13 \times 10^{-4}$ to $1.28 \times 10^{-4}$) and a higher calcination temperature (decreased from $2.13 \times 10^{-4}$ to $2.08 \times 10^{-4}$) led to a lower microstrain in ZnO NPs.

3.1.4 Morphological, particle size, and elemental composition analysis

ZnO NPs showed aggregation under low magnification due to the presence of a weak physical force, while ZnO NPs were well separated and in the nanometre range in high magnification FE-SEM micrographs (48), as shown in Figure 5. ZnO NPs gradually formed a spherical nanostructure at increasing mangosteen leaf aqueous extract concentration, according to the FE-SEM micrographs.

The ZnO NPs calcined at 500°C by using 0.04 g·mL$^{-1}$ mangosteen leaf aqueous extract were analysed by using HR-TEM...
The mean ZnO NP size was 14.21 nm in accordance with the XRD result. Moreover, the structures of ZnO NPs observed from HR-TEM images were similar to those of ZnO NPs observed from FE-SEM micrographs, as both analyses showed a nano-spherical shape. In the EDX spectrum, a weak signal of carbon was detected at 0.25 keV. In ZnO NPs, 37.92% of zinc was detected, while oxygen was found to make up the majority of atoms (62.08%).

3.1.5 Vibrational frequency analysis and surface oxidation state

The micro-Raman spectra and the wide range XPS of ZnO NPs synthesized by using 0.04 g·mL\(^{-1}\) mangosteen leaf aqueous extract and calcined at 500°C are shown in Figures 7 and 8, respectively. The micro-Raman peaks fitted using the Gaussian function of ZnO NPs are shown in Figure 7. The ZnO hexagonal-wurtzite structure is represented by \(A_1 + 2B_1 + E_1 + 2E_2\) as it belongs to the \(\text{P}6_3\text{mc}\) space group, which was in accordance with the XRD results. The polar modes, \(A_1\) and \(E_1\), were split into transverse optical (TO) and longitudinal optical (LO) modes. As a result, the peaks for \(A_1\text{(TO)}\), \(E_1\text{(TO)}\), and \(A_1\text{(LO)}/E_1\text{(LO)}\) were assigned at 387, 414, and 582 cm\(^{-1}\), respectively. The peak at 582 cm\(^{-1}\) was created by an oxygen deficiency-related \(E_2\) multiphonon and resonance mechanism, which was well-resolved. In contrast, the non-polar \(E_2\) mode was located at 436 cm\(^{-1}\), which was related to the oxygen atom and heavier zinc atom vibrations assigned for \(E_{2\text{High}}\) corresponding to the hexagonal-wurtzite ZnO structure. Meanwhile, the \(B_1\) mode was Raman inactive, assigned for \(B_{1\text{(Low)}}\) and \(2E_{2\text{LOM}}\), was located at 287 and 330 cm\(^{-1}\), respectively [48,50,51].
The high-resolution spectra of Zn 2p and O 1s for ZnO NPs are shown in Figure 8(a). In the high resolution spectra of Zn 2p (Figure 8(b)), two prominent peaks were seen that corresponded to the binding energies of Zn 2p_{3/2} and Zn 2p_{1/2}, respectively. This indicated the presence of Zn^{2+}. Furthermore, the difference (23.1 eV) between the two peaks served as a representation of the Zn 2p spin-orbit splitting energy. On the other hand, the high-resolution O 1s spectra in Figure 8(c) revealed two peaks with centres at 526.45 and 528.25 eV, which were attributed to lattice oxygen from ZnO and hydroxyl group oxygen, respectively. These values matched those in previously published works [48,50,52].
3.2 Preliminary in vitro antibacterial study of ZnO NPs, CuO NPs, and ZnO–CuO NCs

ZnO NPs, CuO NPs, and ZnO–CuO NCs exhibited lower MIC values against Gram-positive bacteria (S. aureus and B. subtilis) than those against Gram-negative bacteria (E. coli and K. pneumoniae). The MIC results, as illustrated in Figure 9, were employed to rank the sensitivity of the tested bacteria to the antibacterial effects, arranged from most to least sensitive as follows. Table 3 tabulates the MIC results of the synthesized nanomaterials, and Table 4 shows the MIC values determined in other studies by using green-synthesized ZnO NPs.

- For ZnO NPs:
  \[ S. \text{ aureus} = B. \text{ subtilis} (15.63 \mu g \cdot mL^{-1}) > E. \text{ coli} (62.50 \mu g \cdot mL^{-1}) > K. \text{ pneumoniae} (125.00 \mu g \cdot mL^{-1}) \]
- For CuO NPs:
  \[ B. \text{ subtilis} = E. \text{ coli} (250.00 \mu g \cdot mL^{-1}) > S. \text{ aureus} (250.00 \mu g \cdot mL^{-1}) > K. \text{ pneumoniae} (500.00 \mu g \cdot mL^{-1}) \]
- For ZnO–CuO NCs:
  \[ S. \text{ aureus} = B. \text{ subtilis} (62.50 \mu g \cdot mL^{-1}) > K. \text{ pneumoniae} (125.00 \mu g \cdot mL^{-1}) > E. \text{ coli} (250.00 \mu g \cdot mL^{-1}) \]

4 Discussion

4.1 Physicochemical properties of ZnO NPs

The appearance of bands indicated that the phytochemicals, such as xanthones, flavonoids, and terpenes, found in the aqueous extract of mangosteen leaves [36,38,39] were responsible for the reduction, capping, and
stabilization of ZnO NPs [10,39,55]. The location of $\nu$(Zn–O) shows that the band of metal oxides and hydroxide NPs generally occurred below 1,000 cm$^{-1}$, resulting in interatomic vibrations [48,56]. The $\nu$(Zn–O) from the current study, also reported in other studies, was found in the range of 409–618 cm$^{-1}$ [56–58]. The changes in band intensities of ZnO NPs could be explained by the different interactions between the plant extract functional groups at different controlled parameters. The formation of NPs using natural phytochemicals is still a question. In hypothesis, as a biomolecule model, the xanthone O–H groups would give an electron to electrophile zinc species, causing the hydroxyl group to be reduced to a zinc atom and the electron-deficient zinc ions to be oxidized. In general, the green synthesis of ZnO NPs using the mangosteen leaf extract can occur in three stages: activation, growth, and termination.

Figure 5: FE-SEM micrographs of the mangosteen leaf aqueous extract-mediated ZnO NPs by using different mangosteen leaf aqueous extracts calcined at 400°C and calcined at different temperatures by using 0.04 g·mL$^{-1}$ mangosteen leaf aqueous extract. (a) and (b) show low and high magnifications of FE-SEM micrographs, respectively.
phases. First, the Zn\(^{2+}\) would be released from Zn(NO\(_3\))\(_2\)-6H\(_2\)O when dissolved in the mangosteen leaf extract during the activation stage. The Zn\(^{2+}\) from the divalent oxidation state would reduce to a metallic form in the presence of functional groups from mangosteen leaf extract. During the calcination process, they would oxidize to ZnO NPs immediately due to enhanced chemical reactivity of the bare nanoscale zinc metal surface. ZnO NPs would accumulate and stabilize throughout the growth and termination phases by mangosteen leaf extract phytochemicals [10,30].
The $d \rightarrow d$ transition caused the Zn(NO$_3$)$_2$·6H$_2$O absorption peak to appear, whereas the $\pi \rightarrow \pi^*$ transition of phytochemicals caused the wide absorption peak of the mangosteen leaf aqueous extract [58]. Furthermore, the colour change from light brown to russet observed with the addition of zinc salt demonstrated that Zn$^{2+}$ was reduced to Zn$^0$, which was then oxidized into ZnO during the calcination process. The presence of phytochemicals in the suspension [59] might be the reason for the surface plasmon resonance (SPR)-induced colour change of the suspension [60–62]. SPR occurred by the transfer of electrons from the valence band to the conduction band (O 2$p$–Zn 3$d$) of plant phytochemicals in the aqueous extracts [63], which was initiated by incident electromagnetic radiation [19,23,64] at a particular wavelength [53]. As a result, the absorbance maxima of ZnO NPs were detected, which was consistent with prior experiments utilizing different amounts of plant aqueous extracts [49,65,66] and calcination temperatures [65]. The shift of the ZnO NPs peak to higher wavelengths was due to plant phytochemicals’ non-bonding electron donation to zinc’s unoccupied d-orbital, resulting in simplified electron transitions [34]. The slight drop in the energy bandgap when utilizing a higher plant extract concentration was supported by Demissie et al. [49] and Sato-Robles et al. [66] studies due to the presence of distinct phytochemicals. Furthermore, the dominance of the quantum size effect was aided by the increased crystallinity of the ZnO NPs calcined at higher temperatures [18,67,68]. Moreover, the concentration of localized states in the band structure and width increased with an increase in surface dangling bond counts, leading to a smaller bandgap at higher calcination temperatures [69]. The red-shifted UV-Vis absorption peak in ZnO NPs, which increased from 367 to 373 nm, supports the reduction in energy bandgap at high calcination temperatures [70].

From the XRD spectra, the intensities of (1 0 0), (0 0 2), and (1 0 1) peaks were stronger than other peaks, which showed that they were the preferential crystal planes of ZnO NPs, which was similar to that of Yusoff et al. study [71]. The $P6_3mc$ space group in ZnO NPs was similar to other studies [48,56,57]. Theoretically, a wurtzite crystal ZnO is hexagonal in shape with $a = 3.2960$ Å and $c = 5.2065$ Å as lattice parameters and belongs to the $C_{6v}^3$ or $P6_3mc$, which was similar to the current findings. In the wurtzite hexagonal structure, each anion was surrounded by four cations at the tetrahedron corners and showed a tetrahedral coordination, which was responsible for piezoelectricity and pyroelectricity [15]. As a result, ZnO is in a non-centrosymmetric structure and exhibited sp$^3$ covalent bonding with a polar surface. Therefore, ZnO NPs were most stable and commonly found at ambient conditions as their iconicity was exactly in between covalent and ionic materials [72]. Smaller crystalline sizes at higher mangosteen leaf aqueous concentrations were due to the effectiveness in capping and stabilizing the synthesized NPs [49]. In contrast, larger crystalline sizes at elevated calcination temperatures were caused by the minimization in the interfacial surface energy [44,45], and diffusion of atoms resulted in the disappearance of the grain boundary [73], agglomeration, re-crystallization, aggregation, and growth of particles [69]. The above-mentioned results were similar to those reported in other studies by using different plant extract concentrations and calcination temperatures [49,65,66] in synthesizing ZnO NPs. On the other hand, the aforementioned findings showed that employing a lower concentration of the mangosteen leaf aqueous extract and a higher calcination temperature improved the crystal’s stability. More interfaces can be found in a given volume with a smaller crystalline size, which implies more flaws. As a result, utilizing a higher concentration of the mangosteen leaf aqueous extract and calcining at a lower temperature increased the dislocation density and micro-strain of ZnO NPs [44,45].

The isotropic aggregation at the isoelectric point area [72] caused ZnO NPs to form almost spherical structures with rough surfaces [47], which were then tightly bonded to one another with high affinities [16]. The aforementioned findings were consistent with prior research on the synthesis of ZnO NPs utilizing various plant aqueous extracts [16,57,58] and concentrations [66,74]. Thus, the coarsening and coalescence that occurred with the variations in the mangosteen leaf aqueous extract concentration were evident in the FE-SEM micrographs that showed the alteration in ZnO NPs morphology [75,76]. At high calcination temperatures, ZnO NPs were also more aggregated and porous. Due to the high surface attributes (energy, surface area, reactivity, and tension) [19,23,56], strong attraction
forces [55,77], oxidation of metal oxide NPs [78], and viscous nature of the plant extract [79], it was frequently seen in green-synthesized NPs. Additionally, the escape of gases at higher temperatures during the production of ZnO NPs may have contributed to the pore development [80]. Similar findings were observed when synthesizing ZnO NPs using the hydrothermal technique [52] and Ocimum gratissimum leaf extract [65].

Figure 8: XPS spectra of the mangosteen leaf aqueous extract-mediated synthesized ZnO NPs of (a) overall high-resolution spectra of Zn 2p and O 1s, (b) high-resolution spectra of Zn 2p, and (c) high-resolution spectra of O 1s using 0.04 g·mL⁻¹ mangosteen leaf aqueous extract calcined at 500°C.
The weak signal of carbon was due to the burning of organic materials during calcination, and the soot was left over as impurities [21]. This was not a problem since there were only one or two atoms remaining that showed less interaction [81]. Meanwhile, the oxygen signal in the EDX spectra provided proof that the ZnO NPs were in an oxidized form [22].

4.2 Preliminary in vitro antibacterial activity of ZnO NPs, CuO NPs, and ZnO–CuO NCs

The structural differences between Gram-positive and Gram-negative bacteria are responsible for the variation in susceptibility; Gram-positive bacteria have simpler and thinner cell walls, which makes them more vulnerable to antibacterial
agents [82]. The results indicated that ZnO NPs exhibited the most potent antibacterial activity among the synthesized nanomaterials, possibly attributed to the presence of porosity in ZnO NPs, which provided a high surface-to-volume ratio for effective interaction with the tested bacteria. Additionally, it was suggested by Govindasamy et al. that Zn2+ ions diffused more readily into the medium compared to Cu2+ ions, which could explain the prolonged duration required for Cu2+ to diffuse out from CuO NPs into the medium to exert antibacterial effects [83].

The antibacterial activity of nanomaterials is intricately influenced by their physicochemical properties, including the generation of reactive oxygen species (ROS), surface area, particle size, solubility, and surface charges [84,85]. These unique properties of metal oxide nanomaterials, such as their small particle size, nanomaterial stability, van der Waals forces, hydrophobic interactions, and electrostatic attraction, contribute to their antibacterial activity through various mechanisms [6]. Although a definitive antibacterial mechanism for metal oxide nanomaterials remains elusive, it is widely believed that the antibacterial effects stem from direct interactions with bacterial cell membranes, ROS generation, and the release of free metal ions from nano-metal oxides [1,2,84]. The antibacterial mechanism is illustrated in Figure 10.

Taking ZnO NPs as an example in the first prepared mechanism, these NPs accumulate in the outer membrane or cytoplasm of bacterial cells, potentially triggering the release of Zn2+ ions that attach to biomolecules in the bacterial cell membrane via electrostatic forces. This leads to depolarization of the bacterial cell membrane, resulting in cellular leakage. Moreover, an excess of ROS is induced by the formation of metal ions, which penetrate the bacterial cell membrane, causing denaturation in proteins and lipids, genomic instability, disruption of mitochondrial function, interference with bacterial cell metabolic activity, and ultimately, apoptosis [24,25,86]. Interestingly, particularly in the case of copper-containing nanomaterials, it has been reported that the contribution of dissolved metal ions to antibacterial activity is relatively minor. In the second prepared mechanism, the presence of water and oxygen can only dissolve a small amount of Cu2+ from CuO NPs. However, more free radicals are promoted, and the NPs are converted into metal ions once they enter the acidic lysosomal environment. This process, often referred to as the

<table>
<thead>
<tr>
<th>Nanomaterials</th>
<th>MIC (μg·mL⁻¹)</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO NPs</td>
<td>15.63 ± 0.00</td>
<td>15.63 ± 0.00*</td>
<td>62.50 ± 0.00*</td>
<td>125.00 ± 0.00*</td>
<td></td>
</tr>
<tr>
<td>CuO NPs</td>
<td>250.00 ± 0.00*</td>
<td>125.00 ± 0.00*</td>
<td>125.00 ± 0.00*</td>
<td>500.00 ± 0.00*</td>
<td></td>
</tr>
<tr>
<td>ZnO–CuO NCs</td>
<td>62.50 ± 0.00*</td>
<td>62.50 ± 0.00*</td>
<td>250.00 ± 0.00*</td>
<td>125.00 ± 0.00*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p < 0.05.

Table 4: MIC values determined by using green-synthesized ZnO NPs in other studies

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Crystalline size (nm)</th>
<th>Bacteria</th>
<th>MIC (μg·mL⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelos (pulp)</td>
<td>17.00</td>
<td>Bacillus cereus</td>
<td>8,650.00</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micrococcus luteus</td>
<td>4,680.00–5,080.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>6,650.00–7,090.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>K. pneumoniae</td>
<td>3,840.00–4,340.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter aerogenes</td>
<td>3,990.00–9,090.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>6,760.00–8,020.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas fluorescens</td>
<td>5,190.00–6,090.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>6,870.00–7,860.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella enteritidis</td>
<td>4,450.00–7,220.00</td>
<td></td>
</tr>
<tr>
<td>Brassica rapa (leaf)</td>
<td>41.23</td>
<td>B. subtilis</td>
<td>12.50</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>G. mangostana L. (leaf)</td>
<td>16.99</td>
<td>S. aureus</td>
<td>15.63</td>
<td>Current study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. subtilis</td>
<td>15.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>62.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>K. pneumoniae</td>
<td>125.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: MIC values of the synthesized ZnO NPs, CuO NPs, and ZnO–CuO NCs in different tested bacteria
“Trojan horse mechanism,” promotes the formation of intracellular ROS, disrupting the bacterial cell mitochondrial membrane potential and degrading DNA, ultimately leading to bacterial cell death [25,87,88].

This multifaceted discussion highlights the diverse mechanisms by which metal oxide nanomaterials exert their antibacterial effects and underscores the complexity of their interactions with bacterial cells.

5 Conclusions

In this investigation, we have adeptly employed a sustainable, eco-friendly approach to fabricate ZnO NPs, CuO NPs, and ZnO–CuO NCs utilizing the mangosteen leaf extract. Through a comprehensive suite of analytical techniques encompassing UV-Vis, FT-IR, XPS, Raman spectroscopy, XRD, FE-SEM with EDX, and HR-TEM, we meticulously characterized these nanostructures, offering profound insights into their physical and structural characteristics. Our antibacterial assessments have revealed the remarkable efficacy of ZnO NPs against both Gram-positive (S. aureus and B. subtilis) and Gram-negative bacteria (E. coli and K. pneumoniae), demonstrating the lowest inhibitory concentrations among the tested nanomaterials. While ZnO–CuO NCs and CuO NPs also displayed antibacterial activity, their MICs were marginally higher.

This underscores the potential of ZnO NPs synthesized using the mangosteen leaf aqueous extract as a promising alternative to conventional antibiotics, representing a significant stride in combating antibiotic resistance. In summary, our study not only showcases the successful synthesis of environmentally benign nanomaterials but also underscores their substantial antibacterial efficacy. This advancement holds promise in the development of sustainable and potent antibacterial agents for diverse biomedical applications.

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Ling Shing Wong: conceptualization, writing – review & editing, and funding acquisition; Samar Kumar Guha: conceptualization and funding acquisition; Sinovassane Djearraman: conceptualization; Venkatachalam Rajendran: conceptualization and funding acquisition; Md. Akhtaruzzaman: conceptualization and methodology; Lai-Hock Tey: conceptualization, methodology, writing – review and editing, and supervision.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: All data generated or analysed during this study are included in this published article.

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