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Research article

Green synthesis of copper oxide nanoparticles using christia vespertilionis aerial parts extract: A sustainable approach for antibacterial efficacy assessment

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ABSTRACT

Metal nanoparticles typically exhibit sizes ranging from 1 to 100 nanometers and can be produced by several techniques, such as green synthesis and chemical synthesis. This study focuses on the green synthesis of copper oxide nanoparticles (CuO-NPs) using aerial extract of Christia vespertilionis. The observation of CuO-NPs formation was made based on the variations in colour exhibited by the extract mixture following the introduction of copper (II) sulphate (CuSO4). The synthesised nanoparticles underwent characterization utilising various instrumentation techniques, including UV-Vis spectrophotometry, FTIR, TEM, SEM, EDX, and TGA. The antibacterial activity of these nanoparticles was assessed by using disc diffusion method, against selected Gram-positive and Gram-negative bacteria. The investigation also encompassed an examination of the combined effect of the nanoparticles and the antibiotic Ciprofloxacin on antibacterial activity. In this study, it was noted that the maximum absorbance of the CuO-NPs occurred at a wavelength of 253nm. The functional groups of the CuO-NPs were confirmed through FTIR spectrum. TEM analysis revealed the average particle size of the CuO-NPs as 62.16 nm. Additionally, examination of SEM images revealed that the particles exhibited irregular shapes while maintaining a homogeneous distribution. The nanoparticles exhibited enhanced antibacterial efficacy against Gram-positive bacteria in comparison to the Gram-negative bacteria that were examined. Significant synergistic effect was shown when the CuoNPs were used with the antibiotic against the bacteria tested. In conclusion, the study conducted demonstrates favourable outcomes as an antibacterial agent and recommended to conduct additional research to investigate the mechanism of action and toxicity of this agent.

Keywords: Christia vespertilionis, Copper Oxide nanoparticles, Characterisation, Anti-bacterial efficacy.

INTRODUCTION

Nanotechnology encompasses the scientific, engineering, and technological endeavors that are carried out at the nanoscale, typically ranging from 1 to 100 nanometers. Nanotechnology encompasses the investigation and utilization of particles at the nanoscale, offering potential contributions to diverse scientific domains such as chemistry, biology, physics, materials science, and engineering [1]. Nanoparticles exhibit a high degree of adsorption capacity, which serves as a fundamental basis for their improved performance and enhanced applications [2]. Numerous methodologies exist for the synthesis of nanoparticles, encompassing coprecipitation, hydrothermal synthesis, inert gas condensation, ion sputtering scattering, microemulsion, microwave, pulse laser ablation, sol-gel, sonochemical, spark discharge, template synthesis, and biological synthesis, also known as green synthesis [3]. However, the utilization of green synthesized nanoparticles is regarded as a more cost-effective approach that minimizes environmental pollutants in comparison to existing conventional methods [4].

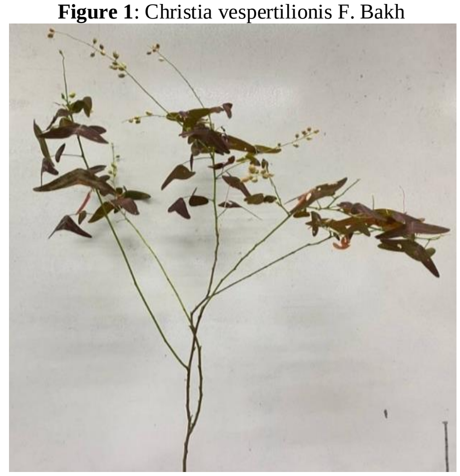
Copper oxide (CuO) is an inorganic compound that exhibits notable physical properties such as high-temperature superconductivity and electron correlation effects [5]. Additionally, it possesses pharmacological attributes, including antioxidant and antibacterial properties. Copper oxide nanoparticles (CuO-NPs) have exhibited efficacy in suspension against select bacterial pathogens, including Escherichia coli and methicillin-resistant Staphylococcus aureus (MRSA), with minimum bactericidal concentrations (MBCs) ranging from 100 g/mL to 5000 g/mL. Based on the research conducted by Ren et al. (2009), the incorporation of CuO-NPs into polymers demonstrated an enhanced ability to eradicate bacterial populations when combined with sub-MBC concentrations of silver nanoparticles. The findings suggest that the release of ions may be necessary to achieve optimal bactericidal effects [6].

CuO-NPs can be synthesized through various methods, including physical, chemical, and green synthesis techniques. The chemical synthesis method entails the utilization of specific chemicals or reagents to facilitate the reduction of copper ions in the process of synthesizing CuO-NPs [7]. In contrast, green synthesis involves the generation of electrons through the utilization of plant extracts to reduce copper salt [8]. Green synthesis techniques frequently employ benign and non-toxic solvents such as water, biological extracts, biological systems, and microwave-assisted synthesis for the production of nanoparticles [9]. Based on the findings of the literature review, there is a lack of existing studies that specifically investigate the synthesis of CuO-NPs derived from Christia vespertiolionis (*C.vespertiolionis*). The plant in question has garnered significant attention from researchers in contemporary times owing to its diverse range of potential pharmacological properties. *C.vespertiolionis* is a member of the Fabaceae (Leguminosae) family and is commonly referred to as Red Butterfly Wing or Mariposa, or alternatively known as "Daun Rerama" in malay [10].

The geographical distribution of this plant encompasses several countries, including Thailand, Vietnam, Cambodia, Indonesia, China, Myanmar, and Malaysia [11]. Although *C.vespertiolionis* has been traditionally used to treat various diseases such as tuberculosis, improve blood circulation, alleviate snake bites, promote bone fracture healing, and alleviate bronchitis and cold symptoms [12], there is a lack of research-based evidence regarding its pharmacological activities [13]. The application of pulverized leaves of this plant onto the affected area is a commonly employed topical treatment for scabies, as well as a method to ameliorate muscle weakness. *C.vespertiolionis* is composed of a diverse range of phytoconstituents, including alkaloids, tannins, saponins, cardenolides, phenolics, cardiac glycosides, flavonoids, and steroids. These phytoconstituents collectively contribute to the plant's anti-inflammatory properties [14]. The picture of the plant was shown in Figure 1.

According to the World Health Organization (WHO), the issue of antimicrobial resistance has emerged as a significant global challenge that poses a threat to humanity. This threat is primarily attributed to the improper utilization of antimicrobial agents, resulting in the emergence of novel resistance mechanisms and the subsequent worldwide dissemination of resistant strains. Consequently, the efficacy of conventional treatments for common infections is being jeopardized [15,16]. The broad utilization of antibiotics in the treatment and prevention of both Gram-positive and Gram-negative bacterial infections, encompassing those acquired in both community and hospital settings, has led to this phenomenon. Previous research has demonstrated the synthesis of CuO-NPs from various plant sources, including Aloe vera, *Calotropis gigantea, Anthemis nobilis, Gloriosa superba, Cinnamomum camphora, Carica papaya, Emblica officinalis, Galeopsidis herba* [17].

Figure 1: Christia vespertilionis F. Bakh



However, there is currently a lack of scientific investigation regarding the production of CuO-NPs from *Christia vespertilionis*. Copper has been recognized for its extensive historical utilization as an antibacterial agent, with CuO-NPs consistently demonstrating notable efficacy against diverse bacterial strains. The observed property is hypothesized to be attributed to the size of the nanoparticles, which could potentially be smaller than the pores of bacteria. This size difference facilitates the nanoparticles' ability to penetrate the cell and exhibit their antibacterial effects [18]. CuO-NPs exhibit antibacterial properties against microorganisms responsible for plant diseases, possess photocatalytic activity for the remediation of dye effluent, and offer various other applications in environmental contexts [19]. Peddi et al. (2021) reported that the CuO-NPs synthesized in their study exhibited significant antimicrobial activity against various common microorganisms, including *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* [20]. According to a study conducted by Azam, Ahmed, Oves, Khan, and Memic (2012), the antibacterial activity of CuO-NPs is influenced by its size. The study found that as the size of CuO-NPs decreases, the values of Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration (MBC) also decrease [21]. The investigation into the green synthesis of CuO-NPs using the medicinal plant *C.vespertiolionis*, which has shown significant potential, has received limited attention thus far. The failure to seize this opportunity holds considerable implications, given the present precarious condition of *C.vespertiolionis* as a species facing threats in Malaysia. The emergence of antibacterial resistance is widely recognized as a significant peril to human existence. Consequently, there is an urgent need for novel methodologies utilizing contemporary technologies. Therefore, the present investigation will center on the production of CuO-NPs using an environmentally friendly approach involving *C.vespertiolionis* aerial extract. Additionally, the aim is to assess the effectiveness of these nanoparticles against specific bacterial strains in terms of their antibacterial properties.

MATERIALS AND METHODS

Plant Materials

C.vespertiolionis was procured from a nursery in Ipoh, Malaysia (Coordinates 4° 35' 50.9244" N and 101° 5' 24.3708" E) and subsequently verified by a taxonomist, after which the herbarium was duly prepared and assigned with specimen number (RCMP/Pharm/Christia Vespertilionis/Herb/X1265). The leaf and stem samples of *C.vespertiolionis* were thoroughly rinsed with running tap water, then fragmented into smaller pieces. Subsequently, these fragments were dried for a designated duration in a shaded environment. After the plants' components were dried, they were processed by grinding them into a coarse powder and subsequently stored in an airtight container for future utilisation.

Preparation of plant extract

The finely ground plant parts were extracted using an aqueous extraction method, wherein distilled water was employed as the solvent. This method incorporated certain adjustments compared to the methodology employed in the previous research conducted by Alhalili (2022) [22]. The aerial components of the desiccated plants were introduced into 100 ml conical flask and added about 35 ml of distilled water. Subsequently, the sample were exposed to a boiling process on a heated surface for a duration of 10 minutes. The solution was allowed to cool for a duration of 10 to 15 minutes. Following this, the solution with aerial parts were filtered using Whatman filter paper No:1, with the resulting filtrate being collected into separate centrifuge tubes. The marc present on the filter paper was subjected for re-extraction to ensure the attainment of maximum extraction. The centrifuge tubes were appropriately labelled and subjected to centrifugation at a speed of 4000 revolutions per minute for a duration of 5 minutes to eliminate any undissolved residues. The liquid portion was collected, appropriately labelled, and stored in a refrigerated environment for subsequent processing in the green synthesis of CuO-NPs.

Green Synthesis of CuO-NPs

About 100 ml of distilled water was mixed with 0.125 g copper (II) sulphate ($CuSO_4$) in a 250 ml conical flask to obtain 5 mM concentration on the metal precursor. 2 ml of the aerial part extract was added, and the conical flask was put on the orbital shaker for 4 days at 120 rpm. The change in colour was monitored to confirm CuO-NPs have been synthesised [22].

Characterisation of CuO-NPs

UV-Visible spectroscopy

About 1ml of green-synthesised CuO-NPs solution were diluted with 2ml of distilled water respectively and the absorbance was measured with Shimadzu UV-1900 in the range of 200 nm - 550 nm against blank solution CuSO4. Then spectrum and absorbance values were observed and recorded [23].

Fourier Transform Infrared (FTIR) Spectroscopy

The identification of the functional groups associated with the green synthesised CuO-NPs was determined by using FTIR in the range of 500 cm^{-1} - 4000 cm^{-1}. The test was conducted on the powder and liquid samples. The FTIR spectrum was observed and recorded [23].

Thermogravimetric Analysis (TGA)

A thermogravimetric analyser Perkin Elmer Pyris 1 was used to examine the thermal stability, thermal activation energies and oxidative stability of the green synthesised CuO-NPs. The powdered sample of leaf extract synthesised CuO-NPs was weighted while heating continuously as an inert gas atmosphere was passed over it. The percentage residue of the sample at various temperatures was observed and recorded for further analysis as the temperature increased [24].

Scanning Electron Microscopy (SEM) & Energy -dispersive X-ray Spectroscopy (EDS)

The surface morphology of the CuO-NPs was investigated through the utilisation of SEM analysis. The liquid sample underwent sonication at a speed of 10,000 revolutions per minute for a duration of 30 minutes. Following this, the supernatant was separated, and the resulting pellet was collected with caution, subjected to a washing process, and subsequently lyophilized. The CuO-NPs in powdered form were coated with conductive layers, specifically gold, using a sputter-coater. Subsequently, the coated CuO-NPs were positioned at the centre of the device. The sample was coated and subsequently underwent scanning SEM analysis using a Hitachi SEM S-4700 instrument, conducted under vacuum conditions. EDS is employed for the purpose of identifying and analysing the elemental composition of nanoparticles (NPs). EDS systems are commonly affixed to the SEM. The identification of elemental Copper and Oxygen was verified through the utilisation of EDX detector [25].

Transmission Electron Microscopy (TEM)

The particle size, shape, and size distribution of the produced CuONPs were analysed using TEM with a Zeiss Libra 120 instrument. A volume of approximately 1 millilitre of the liquid sample containing NPs was subjected to sonication for a duration of 10 minutes. A single droplet of the sonicated solution was deposited onto a carbon grid positioned on filter paper, and subsequently subjected to air drying at ambient temperature for a duration of several minutes. The desiccated sample was placed into a specimen holder and subjected to analysis for the purpose of TEM characterisation [26].

Antibacterial Activity of Green-synthesised CuO-NPs

The antibacterial activity of green synthesised CuO-NPs was evaluated using the nutrient agar disc diffusion method, with a minor adjustment, as described in the study by Dadi et al. (2019) [27]. The present investigation involved the utilisation of three Gram-positive bacteria (Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis) and three Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli, Salmonella) to evaluate the effectiveness of green-synthesized CuO-NPs. The bacteria were cultured using sterile nutrient agar medium plates. The bacteria under investigation were cultured in a nutrient broth medium and subsequently incubated for a duration of 2 hours.

Meanwhile, the stock solution of leaf and stem extracts was prepared through the process of centrifugation, wherein 2 mg of CuO-NPs powder was subjected to centrifugal force in 2 ml of distilled water. Following a duration of 2 hours, the bacteria that had been subjected to incubation were gently collected using a swabbing technique and subsequently transferred onto nutrient agar plates. These plates were then appropriately labelled for identification purposes. Next, the stock solution containing leaf and stem extracts was carefully dispensed onto two separate blank discs. This process was then repeated for various concentrations (20 µL, 40 µL, 60 µL, 80 µL, 100 µL) on the remaining discs. In the present investigation, Ciprofloxacin was employed as the positive control. The procedure was replicated for each bacterial sample, and subsequently, the plates were subjected to a 24-hour incubation period at a temperature of 37 °C to facilitate the observation of microbial proliferation. The plates were examined to determine the zone of inhibition, and the diameter was measured in centimetres.

Synergistic Antibacterial Activity of CuO-NPs and Ciprofloxacin

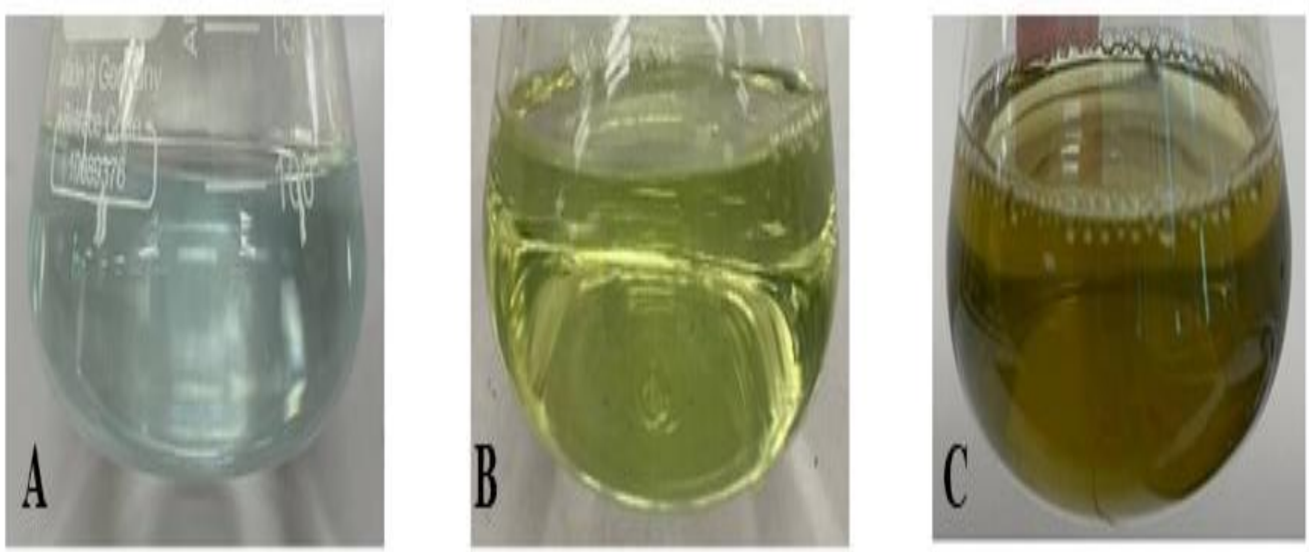
The synergistic antibacterial activity of CuO-NPs and Ciprofloxacin was tested by referring to the method in the study by Nene and Tuli (2019) with a slight modification as required in this study, using a disc diffusion method similar to the one used in the antibacterial activity test [28]. This test involved the combination of the green-synthesised CuO-NPs against two Gram-positive bacteria (Bacillus cereus, and Staphylococcus aureus). 40 µL.of the green-synthesised CuO-NPs was pipetted onto the Ciprofloxacin disc and the disc was placed in the centre of each bacteria plate. The plates then were incubated at 37 °C for 24 hours to observe the microorganism's growth which is indicated by the zone of inhibition. The zone was observed, and its diameter was measured in centimetres.

RESULTS AND DISCUSSION

Green synthesis of CuO-NPs

The change in colour of the metal precursor $CuSO_4$ upon the addition of *C.vespertiolionis* aerial extract is an indication of reducing $CuSO_4$ to CuO-NPs [22]. This change confirms the green synthesis of CuO-NPs as the Cu^+ ions are reduced resulting in the colour change of the solution as shown in Figure 2.

Figure 2: A: Metal precursor $CuSo_4$ in distilled water; B: Metal precursor + aerial part extract; C: CuO-NPs solution.



Characterisation of CuO-NPs

UV-Visible spectroscopy analysis

The formation of the green-synthesised CuO-NPs was confirmed by running the UV-Visible spectroscopy after the colour of the solution was found to change from the original colour. Figure 3 a shows the UV-Vis absorption spectrum of green synthesised CuO-NPs with a maximum peak at 253 nm. Figure 3 A shows UV spectrum of the aerial extract synthesised CuO-NPs. Despite the slight differences in the highest peak value of the samples, all these values are valid to prove the formation of CuO-NPs as stated in a previous study by Sarkar et al. (2020) and Mobarak et al. (2022) which the absorbance peak for CuO-NPs was found roughly to be in 200 – 300 nm of the range [29,24]. It is also documented in the study by Mobarak et al. (2022), the extract solution's colour changes from its initial blue to a dark brownish hue, indicating that CuO-NPs' ability to excite the surface plasmon resonance (SPR) phenomenon and could be another indication of CuO-NPs formation. This phenomenon usually happened in the ranges from 200 nm to 300 nm [24].

Fourier Transform Infrared (FTIR)

FTIR analysis was carried out on the green synthesised CuO-NPs to determine their stretching and vibrating modes as well as to determine the presence of the functional groups associated with the nanoparticles formed. Figure 3 B shows the FTIR result for stem extract synthesised CuO-NPs in powder form where the peaks were observed at wavelengths of 3123 cm $^{-1}$, 1633 cm $^{-1}$, 1061 cm $^{-1}$, and 613 cm $^{-1}$. As shown in Figure 4.10, the FTIR measurements for a liquid sample of stem extract synthesised CuO-NPs, the peaks can be seen at 3315 cm $^{-1}$, 1635 cm $^{-1}$, and 455 cm $^{-1}$. According to Alhalili (2022), the presence of the CuO-NPs can be confirmed by the peak shown at 594 cm $^{-1}$ which is associated with Cu-O vibrations [22].

Transmission Electron Microscopy (TEM) analysis

The TEM results revealed that the CuO NPs exhibited an average size distribution ranging from 82.75 nm to 33.45 nm. The average size of nanoparticles was determined to be 62.16 nm. According to Vichare et al., it was stated that CuONPs with varying particle sizes between 20 and 95 nm exhibited notable antibacterial activity against harmful bacteria such as Escherichia coli [30]. TEM image was shown in Figure 3.C.

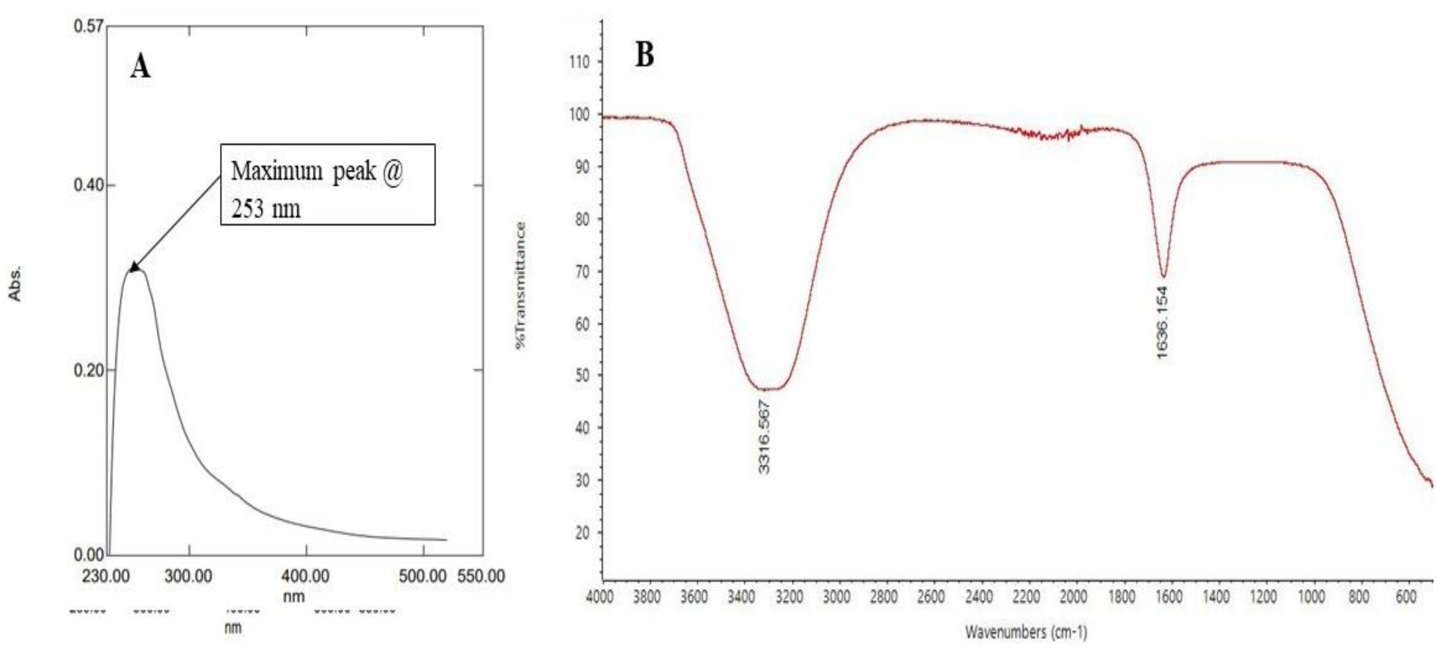
Scanning electron microscopy (SEM) & Energy Dispersive X-ray (EDX) analysis

The scanning electron microscopy (SEM) image indicated that the copper oxide nanoparticles (CuO NPs) exhibited an irregular shape while maintaining a consistent level of homogeneity as shown in Figure 3.D. The findings were reinforced by a study conducted by Selvaraj et al. (2019), which similarly demonstrated the uniform dispersion of CuO nanoparticles [31]. The EDX examination revealed that the CuO NPs had a copper (Cu) element composition of 99.87% by weight as shown in Figure 3.E. The findings of this study indicate that the nanoparticles under investigation were composed solely of copper and a trace amount of gold (Au) was detected as an impurity in this examination that might be encountered due to sample loading process for the study.

Thermogravimetric Analysis (TGA)

The TGA result for the green-synthesised CuO-NPs was observed for its percentage residue after heating to a certain temperature ranging of 50 °C - 750 °C. Figure 3.E represents the TGA curve of the degradation of protein at four different stages on the green-synthesised CuO-NPs at their respective temperature. About 21.61% weight loss was recorded on the sample at the temperature of 109.52 °C. According to Mobarak et al. (2022), the weight loss at the first stage might be due to the removal of any volatile substances that may have been present by physiosorbed H_2O, such as deionized water, moisture, ethanol, or surface water. At a temperature of 197.22 °C, the second stage of weight loss was observed, and the calculated percentage of weight loss was around 14.67%. The release of the remaining volatile organic chemicals that may present due to incomplete conversion of $CuSO_4$ to CuO-NPs and water molecules that might not have been eliminated in the first stage is believed to be responsible for this weight loss [24]. The greatest weight loss was observed at 296.23 °C with the loss of 24.23%, final stage recorded 5.45% weight loss in the sample at 747.68 °C. In this study, the total weight loss of the green-synthesised CuO-NPs was 65.96% up to a studied temperature of 750 °C that proves the green synthesised CuO-NPs shows good thermal stability even at higher temperatures. Comparing to previous findings from Mobarak et al. (2022), it shows their synthesised CuO-NPs have moderate thermal stability with 34.83% of weight loss at a temperature up to 1000 °C [24].

Figure 3: Characterisation of CuONPs, A: UV-Visible spectrum; B: FTIR spectrum

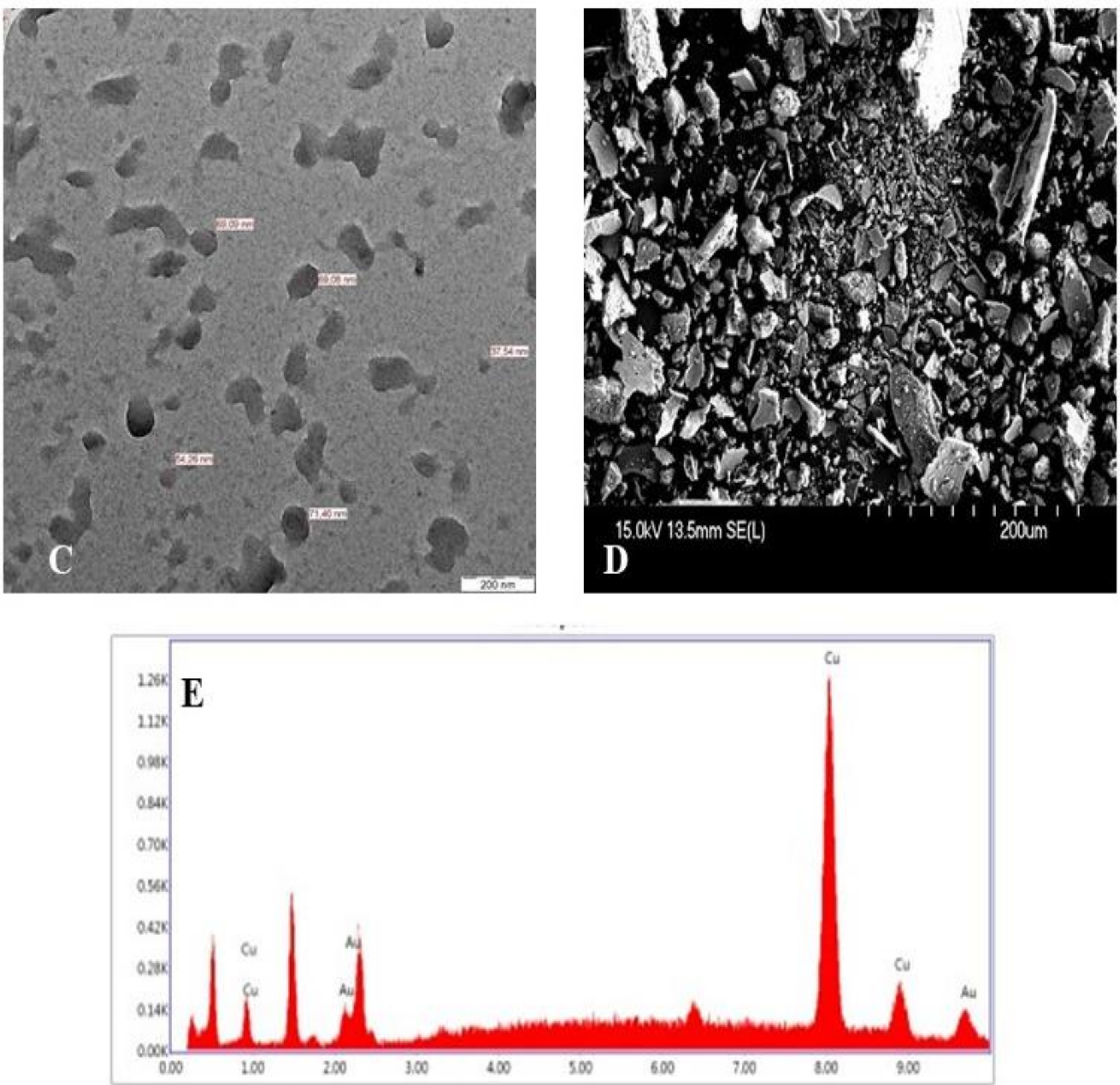


Antibacterial efficacy of green synthesised Cuo-Nps

Antibacterial activity of Green-Synthesised Cuo-Nps against gram-positive and gram-negative bacteria

Antibacterial activity of the green-synthesised CuO-NPs was studied against 3 selected Gram-positive (*Bacillus cereus*, *Staphylococcus aureus* (ATCC 33862), *Staphylococcus epidermidis*) and 3 selected Gram-negative (*Pseudomonas aeruginosa* (ATCC 15442) , *Escherichia coli* (ATCC8739) , *Salmonella spp.*) bacteria by using a nutrient agar disc diffusion method. Ciprofloxacin which is a broad-spectrum antibiotic in the class of fluoroquinolone was used as a positive control in this study. This antibiotic is used to treat a variety of bacterial illnesses, including typhoid fever, urinary tract infections, skin infections, respiratory tract infections, and infections of the bones and joints [32]. Table 1 shows the result of green synthesised CuO-NPs against tested bacteria. The evaluation was assessed measuring the zone of inhibition observed surrounding the disc on the inoculated plates. Absence of zone indicates the absence of antibacterial effect. The minimum inhibitory concentration (MIC) for the green synthesised CuO-NPs against *Bacillus cereus* was 20 µL, 40 µL for *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Pseudomonas aeruginosa,* and 60 µL for *Escherichia coli*. In contrast, Ciprofloxacin seems to be less against *Escherichia coli* compared to other tested bacteria. To support this statement, according to a study conducted by Mandal (2012), stated that 73% of all *Escherichia coli* isolates were found to be resistant to Ciprofloxacin. in his previous reported study [33].

Figure 3: C: TEM image; D; SEM image; E: EDX graph



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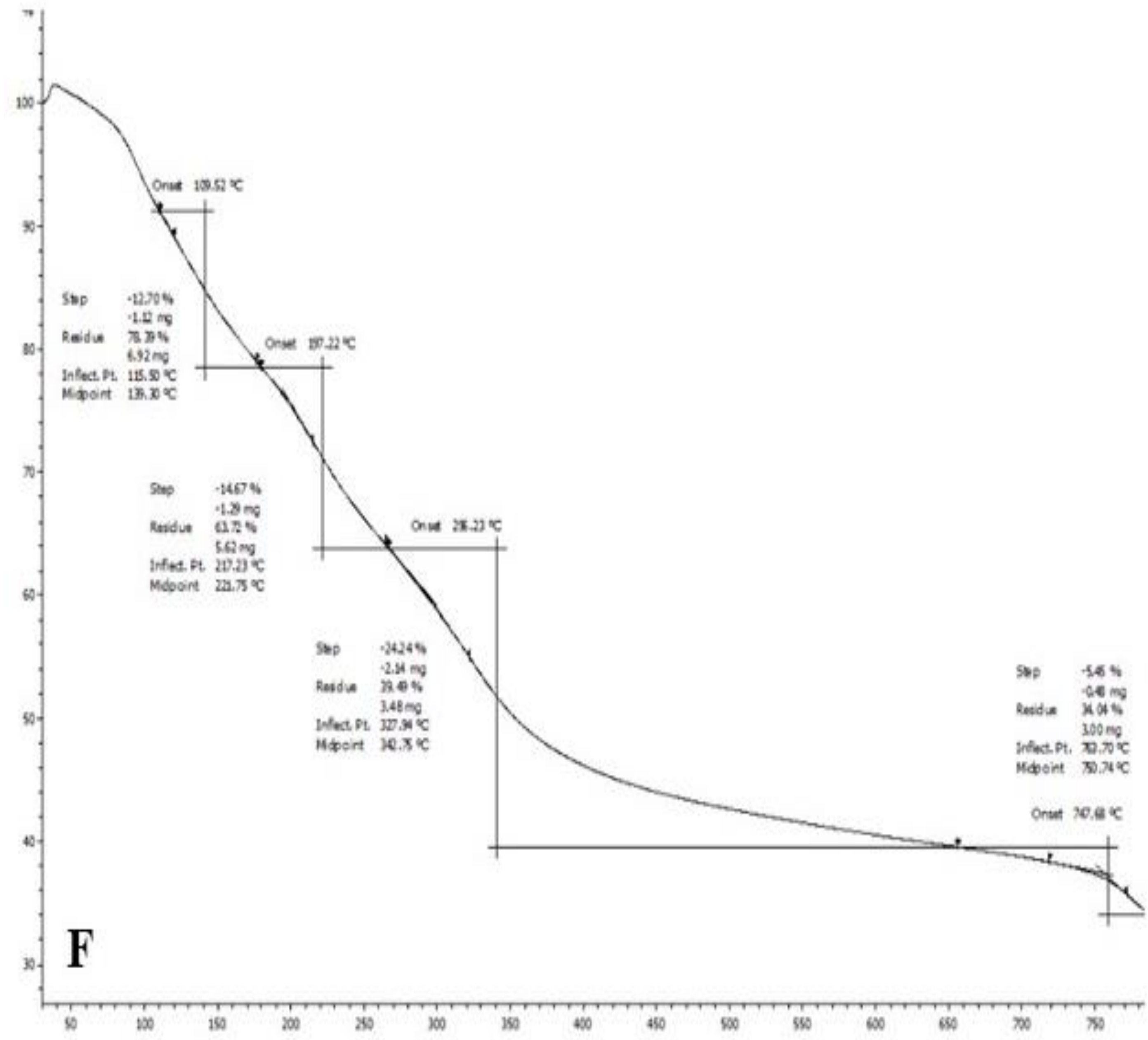
From this study, the results shows that the green-synthesised CuO-NPs were effective to prevent the growth of both Gram-negative and Gram-positive bacteria but the susceptibility of the antibacterial activity of these nanoparticles was more to Gram-positive bacteria. The structure of Gram-positive and Gram-negative bacteria differs, which may be the cause of this outcome. It is believed that Gram-negative bacteria have an exterior layer membrane that shields them from the outside environment. This membrane is thought to be very selective to the foreign materials going through it, making it more difficult to penetrate [34] which might be solved by increasing the concentration of the CuO-NPs.

Table 1: Antibacterial efficacy of CuO-NPs synthesised from leaf extract.

Bacteria	Diameter zone of inhibition (cm)					
	Positive control (Ciprofloxacin)	Concentration of the sample (µL)				
		20	40	60	80	100
B. cereus	2.8	0.7	0.7	0.9	1.0	1.2
S. aureus	2.9	0.3	0.6	0.7	0.9	1.0
S.epidermidis	3.0	0.3	0.5	0.7	0.8	0.9
P.aeruginosa	3.0	-	0.3	0.4	0.6	0.7
E. coli	2.5	-	-	0.3	0.4	0.6
Salmonella spp.	3.4	-	-	-	-	-

Figure 3: F: DSC spectrum



Synergistic Antibacterial Activity of CuO-NPs and Ciprofloxacin

The production of hydroxyl radicals, modification of cellular defence mechanisms, and an anti-biofilm potential were said to be the factors contributing to synergistic activity. Combining antibiotics and nanoparticles against bacteria was confirmed by Elmahallawy et al.

(2020) to increase the efficacy of the antibiotics as an antibacterial agent rather than using the antibiotics alone [35]. Thus, the synergistic antibacterial activity of CuO-NPs and Ciprofloxacin were studied against gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) to evaluate their significant contribution as a potential antibacterial agent. The study results showed a phenomenal increase in the zone of inhibition on the two tested bacteria with 40 µL of green-synthesised CuO-NPs and standard antibiotic. Table 2. Show the results of synergistic antibacterial efficacy on tested microorganism. From the results observed it can be understood that the green synthesised CuO-NPs potentially increases the activity of the standard ciprofloxacin.

Table 2: Synergistic antibacterial activity of CuO-NPs and Ciprofloxacin.

Sample(ml)	Zone of Inhibition (cm)			
	Bacillus cereus		*Staphylococcus aureus*	
	Ciprofloxacin	Ciprofloxacin + CuO-NPs	Ciprofloxacin	Ciprofloxacin + CuO-NPs
Green synthesised CuO-NPs	2.8	3.1	2.9	3.5

CONCLUSION

In conclusion, this study successfully synthesized CuO-NPs using *Christia vespertilionis*, a medicinal herb, through a green approach. The investigation provided valuable insights into CuO-NPs' characteristics and their antibacterial potential, both independently and in combination with standard antibiotics. Notably, CuO-NPs displayed considerable efficacy against certain Gram-positive bacteria, while their effectiveness against Gram-negative strains requires further scrutiny. In summary, this study represents a noteworthy contribution to green synthesis and nanoparticle-based antibacterial research. While promising, it underscores the need for continued investigation and development, holding potential for CuO-NPs as eco-friendly tools against bacterial infections. This research paves the way for sustainable technological advancements in the fight against pathogens.

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