

ANTIMICROBIAL POTENTIAL OF MULTISCALE POLYMERIC NANOSCAFFOLDS LOADED WITH GREEN SYNTHESIZED CUO NANOPARTICLES FROM *POCOCKIELLA VARIEGATA* EXTRACT

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ABSTRACT

This study developed a green-synthesized copper oxide Nanoparticles (CuO NPs)-loaded starch/PVA nanoscaffold system with enhanced antimicrobial properties. UV-Vis spectroscopy confirmed the presence of bioactive compounds in *Pocockiella variegata* algal extract (peak at 267 nm) and revealed an atypical surface plasmon resonance for CuO NPs at 292 nm, suggesting unique NP characteristics. Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analyses verified successful CuO NP formation and their stable integration into nanoscaffolds through hydrogen bonding and structural interactions. scanning electron microscopy (SEM) imaging showed uniformly distributed CuO NPs within porous, bead-free nanofibers, while Thermogravimetric analysis (TGA) demonstrated improved thermal stability (degradation onset at 262.78°C vs. <250°C for pure scaffold). Dynamic light scattering (DLS) indicated moderate colloidal stability (zeta potential: -10.03 mV), highlighting the need for surface optimization. Antimicrobial assays revealed superior activity of CuO NPs-loaded scaffolds, with inhibition zones of 30±0.16 mm (*E. coli*), 28±0.18 mm (*K. pneumoniae*), and 25±0.35 mm (*Penicillium*), outperforming standalone CuO NPs and algal extract. These results demonstrate the scaffold's potential for biomedical applications, particularly in combating drug-resistant pathogens. Future studies should focus on enhancing NP dispersion and long-term stability for clinical translation.

KEYWORDS: *Pocockiella variegata*; Green synthesis; CuO NPs; Starch/PVA nanoscaffolds; Antimicrobial activity.

1. INTRODUCTION

The escalating burden of antimicrobial resistance (AMR) worldwide has catalyzed an urgent search for novel strategies to counteract microbial infections [1]. Traditional antibiotics are progressively becoming ineffective against a broad spectrum of pathogens, leading to prolonged illness, increased mortality, and substantial economic losses [2]. In response, nanotechnology has emerged as a transformative approach to addressing microbial resistance through the development of nanoscale materials with potent antimicrobial properties [3]. Among the various metal-based nanoparticles (NPs) studied to date, copper oxide NPs (CuO NPs) have attracted considerable attention due to their cost-effectiveness, biocompatibility, environmental sustainability, and broad-spectrum antimicrobial efficacy [4].

Conventional methods of synthesizing CuO NPs typically involve the use of hazardous chemicals, extreme reaction conditions, or energy-intensive processes, all of which contribute to environmental degradation and limit their application in biomedical and pharmaceutical settings [5]. To overcome these limitations, green synthesis methodologies have been developed as sustainable alternatives [6]. These methods utilize plant extracts, microbial cultures, or algal biomass as reducing and stabilizing agents to facilitate NP formation under mild conditions [7]. Marine macroalgae, in particular, are rich reservoirs of bioactive metabolites such as polysaccharides, polyphenols, proteins, and flavonoids, all of which can participate in metal ion reduction and NP capping [8]. These naturally occurring compounds not only ensure the stability and biocompatibility of the resulting NPs but may also impart additional biological functionalities [9].

The marine macroalga *Pocockiella variegata*, endemic to coastal regions such as Rameswaram, Tamil Nadu, India, remains largely unexplored for its nanobiotechnological potential [10]. This species is known to harbor various secondary metabolites with antioxidant, antimicrobial, and anti-inflammatory properties, which make it a promising candidate for NP biosynthesis [11]. Employing *P. variegata* extract as a bioreductant aligns well with the principles of green chemistry, as it avoids the use of synthetic additives while producing biologically active nanomaterials [12]. In this study, we leverage the rich phytochemical profile of *P. variegata* for the eco-friendly synthesis of CuO NPs through an ultrasonic-assisted aqueous extraction method [13].

The development of functional nanocomposite scaffolds incorporating metal oxide NPs represents a promising direction in the field of tissue engineering, wound healing, and antimicrobial therapies [14]. Electrospinning—a versatile and cost-effective technique—allows for the fabrication of nanofibrous scaffolds that closely mimic the architecture of the natural extracellular matrix (ECM) [15]. These scaffolds offer large surface area-to-volume ratios, high porosity, and the ability to integrate bioactive compounds, making them ideal platforms for controlled drug release and antimicrobial action [16]. Polyvinyl alcohol (PVA), a hydrophilic, biocompatible, and biodegradable synthetic polymer, is widely used in electrospinning due to its excellent film-forming and mechanical properties [17]. However, PVA alone may lack sufficient bioactivity or degradation control for certain biomedical applications [18]. To address this, starch—a natural polysaccharide derived from agricultural sources—is often blended with PVA to enhance biocompatibility, promote cellular interactions, and adjust degradation rates [19]. The inclusion of starch also adds a sustainable and economical dimension to scaffold design. When biosynthesized CuO NPs are embedded into starch/PVA electrospun nanofibers, the resulting composite materials combine mechanical robustness, biodegradability, and enhanced antimicrobial efficacy [17]. Several recent studies have demonstrated that CuO NPs exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacterial strains as well as fungi [20]. These effects are mediated through multiple mechanisms, including disruption of microbial membranes, induction of oxidative stress via reactive oxygen species (ROS) generation, and interference with enzymatic and genetic functions [21]. When integrated into nanofibrous scaffolds, these NPs provide a localized antimicrobial effect while minimizing systemic toxicity [22]. Moreover, electrospun fibers allow sustained release of NPs at the wound site or infected area, enhancing therapeutic outcomes without the need for repeated administration [23].

The ultrasonic-assisted extraction method employed in this study not only enhances the yield of bioactive compounds from *P. variegata* but also preserves the functional integrity of these molecules [24]. Sonication facilitates cell wall disruption, leading to efficient solubilization of intracellular components [25]. The extracted phytochemicals are then used as reducing and stabilizing agents for the synthesis of CuO NPs, eliminating the need for external chemical additives [26]. Subsequent characterization of the NPs is carried out using an array of analytical techniques including UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). These techniques help confirm the structural integrity, morphology, and crystallinity of the NPs [27]. The fabricated CuO NP-loaded starch/PVA nanofibrous scaffolds are also subjected to a comprehensive set of physicochemical evaluations [28]. Thermogravimetric analysis (TGA) is employed to assess the thermal stability and degradation profile of the nanocomposites [29]. Particle size and zeta potential measurements are conducted to determine colloidal stability and ensure consistent NP dispersion within the scaffold matrix [30]. These properties are crucial in predicting the behavior of the scaffolds under physiological conditions and their interactions with biological tissues [31]. To evaluate the antimicrobial efficacy of the synthesized materials, an agar well diffusion assay is performed against a panel of clinically relevant bacterial and fungal pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *Aspergillus niger*, and *Penicillium* spp. [32]. The comparison of antimicrobial effects among the algal extract, CuO NPs, and nanofiber scaffolds provides insight into the synergistic potential of the composite materials [33]. Statistical analyses are performed to ensure the reliability and reproducibility of the experimental outcomes, with significance determined through ANOVA and post hoc comparisons [34].

This study aims to contribute to the growing field of green nanotechnology and biomedical materials by demonstrating a sustainable, efficient, and scalable approach to NP synthesis and scaffold fabrication [35]. By integrating biosynthesized CuO NPs into starch/PVA electrospun nanofibers, we propose a multifunctional nanocomposite that can serve as an effective antimicrobial platform [36]. The use of *P. variegata* as a novel bioreductant not only enhances the bioactivity of the NPs but also highlights the untapped potential of marine macroalgae in nanomaterial development [8].

2. MATERIALS AND METHODS

2.1. Materials

The experimental materials included *P. variegata* macroalgae, harvested from the coastal area of Rameswaram, Tamil Nadu, India. Copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was acquired from Sigma-Aldrich and utilized for synthesizing CuO NPs. PVA with a molecular weight of 115,000 served as the electrospinning polymer matrix, while starch sourced from a local vendor was incorporated into the nanofiber scaffolds. Analytical-grade reagents and solvents, such as ethanol, Milli-Q water, and other required chemicals, were procured from Merck and Sigma-Aldrich. Electrospinning was performed using a high-voltage power source (HOLMARC HO-NFES-040) coupled with a syringe pump to fabricate PVA/CuO NPs nanofibers. Analytical instruments employed included a UV-Vis spectrophotometer (VIS SPEC V-730), Fourier-transform infrared (FT-IR) spectrometer (Spectrum Two, PerkinElmer), X-ray diffractometer (Bruker D8 Advance), and scanning electron microscope (SEM JSM-IT800 NANO). Particle size and zeta potential measurements were carried out using the Malvern Zetasizer Ultra, while thermal stability was evaluated through TGA using the PerkinElmer TGA 8000.

2.2. Collection of marine macroalgae

Specimens of the marine macroalga *P. variegata* were collected from the Rameswaram shoreline in Tamil Nadu, India. The collected *Pocockiella variegata* was taxonomically identified and authenticated by Dr V. Veeragurunathan, CSIR-CSMCRI-Marine Algal Research Station, Mandapam Camp 623519, Tamil Nadu, India. A voucher specimen was prepared and deposited at the Herbarium of the CSIR-CSMCRI-Marine Algal Research Station, under the accession number: SI-2025-RMM-024. This authentication ensures botanical accuracy and standardization of the study material.

The algae were initially rinsed with tap water to eliminate surface debris, followed by washing with distilled water to ensure removal of residual contaminants. Around 100 grams of the cleaned algal sample were cut into small pieces by hand and left to air-dry under room conditions for a total of 14 days. Upon complete dehydration, the algae were ground into a uniform powder using a mechanical grinder, preparing the material for extraction and further experimentation.

2.3. Ultrasonic-assisted extraction

To obtain the extract, 2 g of the powdered *P. variegata* were mixed with 50 mL of Milli-Q water in a 100 mL beaker. This mixture underwent ultrasonic-assisted extraction using an ultrasonic cleaner (LABQUEST by Borosil, Serial No. 941032329018) maintained at 60°C for 45 minutes to facilitate enhanced bioactive compound release. The mixture was then filtered through Whatman No. 1 filter paper under sterile laboratory conditions. The resulting filtrate was transferred into a clean 50 mL beaker and stored at 10°C. For conversion into powdered form, the extract was freeze-dried using a lyophilizer, rendering it suitable for subsequent analysis [37].

2.4. Biosynthesis of CuO NPs

To synthesize CuO NPs via green chemistry, 50 mL of a 0.1 M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution was combined with 10 mL of the prepared aqueous algal extract (dissolved from 10 mg dried extract in 10 mL Milli-Q water) in a 5:1 ratio. This solution was stirred at 650 rpm and maintained at 60°C for 2 hours on a magnetic stirrer. A visible color transition from blue to pale bluish-green indicated CuO NP formation. The product was subsequently washed three times with double-distilled water while maintaining identical stirring and thermal conditions to eliminate unreacted constituents. The cleaned NP suspension was freeze-dried for 48 hours, producing powdered CuO NPs for further analyses and applications [38].

2.5. Fabrication of CuO NPs-loaded starch/PVA nanofiber scaffolds

Electrospun polymer nanofibers were developed by first preparing a 15% (w/w) fully hydrolyzed PVA solution in double-distilled water. Into this solution, 100 mg each of starch and biosynthesized CuO NPs were dispersed, and the mixture was stirred rigorously at 50°C for 2.5 hours to achieve uniform blending. Electrospinning was executed using the HOLMARC HO-NFES-040 setup with an HMPSKV30 power supply (0–30 kV, 0.5 mA). The polymer mixture was loaded into a syringe with a 0.84 mm needle, and nanofibers were produced under optimized parameters: 11.5 kV voltage, 12.3 cm needle-to-collector gap, and 0.4 mL/h flow rate. Electrospinning was conducted at room temperature for 6–8 hours, resulting in homogenous nanofiber mats [17, 39, 40].

2.6. Physicochemical characterization

2.6.1. UV–visible spectroscopy

The optical properties of the marine algal extract, synthesized CuO NPs, and CuO NPs-integrated starch/PVA nanofibers were examined using UV–Visible spectroscopy. A VIS SPEC V-730 UV–Vis spectrophotometer was used to record absorbance spectra over the 200–800 nm range. Deionized water was employed as a blank reference for all measurements [41].

2.6.2. FT-IR Spectroscopy analysis

FT-IR analysis (PerkinElmer Spectrum Two, ATR mode) was conducted to study the functional groups in the macroalgae extract, green-synthesized CuO NPs, and CuO NP-loaded starch/PVA nanofibers. Spectra were acquired in the 4000–400 cm^{-1} range with a resolution of 4 cm^{-1} . Each sample was scanned 64 times to enhance signal clarity and ensure accurate molecular profiling, helping identify interactions among the scaffold components [42].

2.6.3. XRD analysis

The crystalline properties of CuO NPs and CuO-loaded starch/PVA nanofibers were determined via XRD using a Bruker D8 Advance diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$), operated at 40 kV and 40 mA. Diffraction data were gathered in the 2θ range of 5° to 90°, with a step interval of 0.029649°, enabling phase identification and crystallite size estimation [43].

2.6.4. SEM analysis

SEM imaging was used to investigate surface morphology and fiber structure of both the green-synthesized CuO NPs and the PVA/starch nanofibers embedded with them. A JEOL JSM-IT800 NANO SEM was employed for high-resolution analysis. Prior to imaging, samples were gold-coated using a sputtering unit to enhance conductivity and optimize image resolution [44].

2.6.5. TGA

Thermal degradation behavior of CuO NP-infused starch/PVA nanofibrous scaffolds was studied using a TGA 8000 analyzer (PerkinElmer). Approximately 5 mg of each sample was placed in aluminum crucibles and heated from room temperature to 550°C at a ramp rate of 15°C/min under nitrogen flow. Mass loss patterns were tracked to assess decomposition stages and thermal resilience of the materials [45].

2.6.6. Particle size and zeta potential analysis

The physicochemical stability and size of the biosynthesized CuO NPs were evaluated using a Malvern Zetasizer Ultra system. Dynamic light scattering (DLS) provided data on particle size distribution, while zeta potential values were measured to determine colloidal stability through electrophoretic mobility. These analyses ensured a comprehensive

understanding of NP behavior in suspension [46]. The complete set of characterization results has been deposited in Mendeley Data under the DOI: 10.17632/6tddv74p8k.1.

2.7. Antimicrobial activity

The agar well diffusion method evaluated the antimicrobial effect of *P. variegata* extract (50 µg/mL), CuO NPs (50 µg/mL), and starch/PVA electrospun nanoscaffolds (50 µg/mL) on *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Citrobacter koseri*, *Aspergillus niger*, and *Penicillium* species. Bacterial and fungal suspensions were adjusted to 0.5 McFarland turbidity and spread on Mueller-Hinton agar (bacteria) and Sabouraud dextrose agar (fungi). Wells (6 mm diameter) were punched, and 50 µL of test samples (50 µg/mL), positive controls (ciprofloxacin/voriconazole), were added. Plates were pre-diffused (30 minutes), then incubated at 37°C (24 hours, bacteria) and 28°C (48 hours, fungi). Activity was measured by inhibition zone diameters (mm), with larger zones indicating higher efficacy. Tests were performed in triplicate for accuracy [47, 48].

2.8. Statistical evaluation

Statistical interpretation of the experimental findings was carried out using SPSS version 25.0 and GraphPad Prism 9.0 software. Data from triplicate experiments were expressed as mean ± standard deviation. One-way ANOVA followed by either Tukey's or Dunnett's post-hoc tests was utilized to compare groups, setting significance at $p < 0.05$. Pearson's correlation coefficient was used to assess associations between variables, while data normality was checked using the Shapiro–Wilk test. If necessary, non-normal data were transformed logarithmically. All results were analyzed within a 95% confidence interval to ensure reliability [49].

3. RESULTS

3.1. UV–visible spectroscopy

The UV–Visible spectral analysis of the macroalgae extract (Fig. 1) showed a marked absorbance peak at 267 nm (absorbance = 0.4035), typically associated with UV-absorbing compounds such as phenolics or aromatic conjugates. Across the 200–800 nm range, absorbance values varied from 0.3285 to 0.4035. The limited absorption within the visible light spectrum (400–700 nm) pointed to a reduced chlorophyll content, likely due to pigment degradation or extraction processes. In contrast, the spectrum of CuNP dilution (Fig. 1) revealed a surface plasmon resonance (SPR) peak at 292 nm (absorbance = 0.70716), an unusual shift from the standard CuNP range (570–600 nm), possibly due to nanoscale size, oxidative changes, or stabilizer interactions. The absorption spanned 0.31409 to 0.70716, with a rapid drop after the peak, suggesting NP monodispersity and minimal clumping.

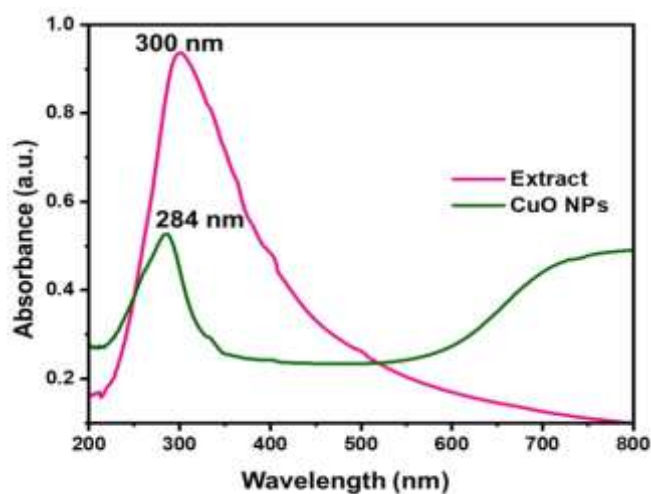


Fig. 1. UV–Visible spectral profiles of algal extract and CuO NPs.

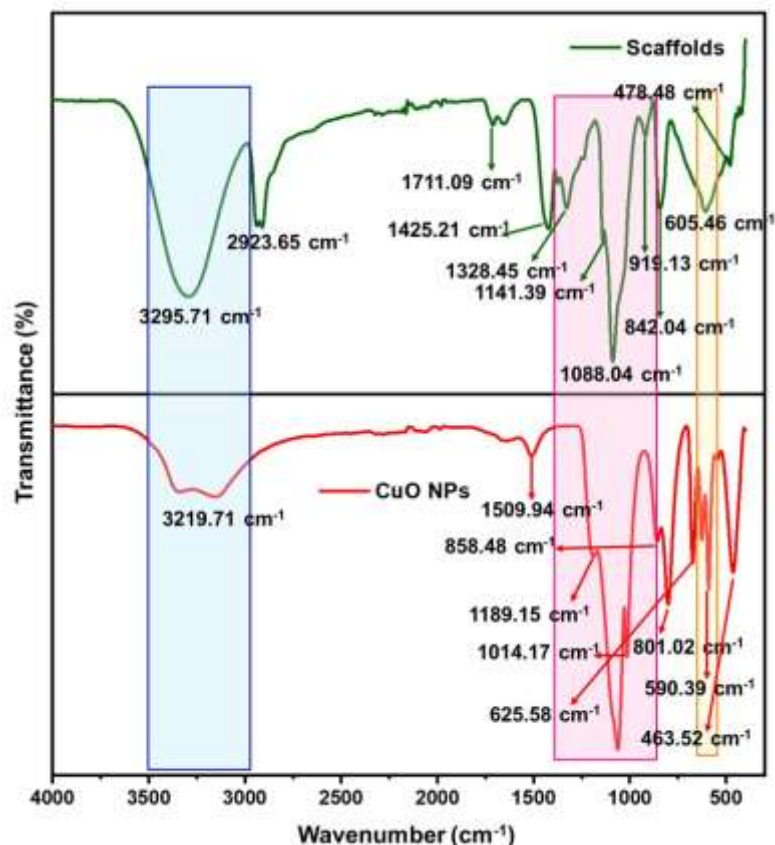


Fig. 2. FTIR spectra of CuO NPs and their incorporation into nanoscaffolds.

3.2. FTIR spectroscopy analysis

FTIR spectral evaluation of the biosynthesized CuO NPs (Fig. 2A) indicated significant vibrational bands. These included a broad -OH/N-H stretch around 3219.71 cm^{-1} , peaks at 1509.94 cm^{-1} corresponding to aromatic stabilizers, and Cu-O bonds visible between $672\text{--}463\text{ cm}^{-1}$, confirming successful NP formation. The starch/PVA nanoscaffolds presented distinct peaks (Fig. 2A), including -OH (3295.71 cm^{-1}), C-H stretching (2923.65 cm^{-1}), and C-O-C linkages ($1141\text{--}1088\text{ cm}^{-1}$) from the biopolymer backbone. Overlapping bands near $605\text{--}478\text{ cm}^{-1}$ indicated embedded Cu-O bonds, verifying NP integration. A shift in the -OH region suggested hydrogen bonding between CuO particles and the scaffold, enhancing structural stability. Additionally, a peak at 1711.09 cm^{-1} was observed, likely denoting C=O stretching due to potential oxidation of PVA or its interactions with CuO NPs.

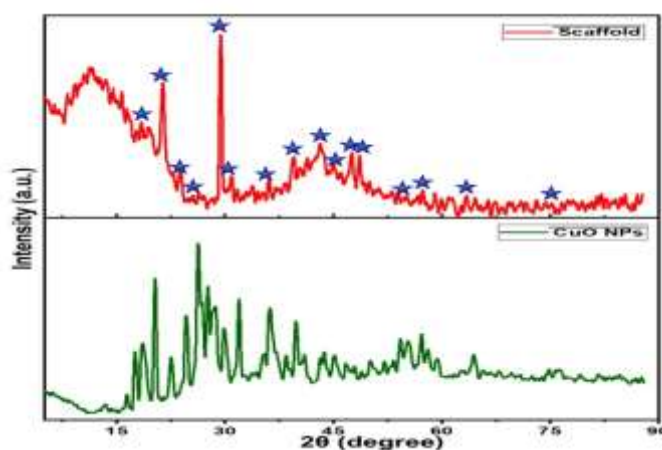


Fig. 3. XRD patterns of synthesized CuO NPs and CuO-loaded scaffolds.

3.3. XRD analysis

XRD characterization of the CuO NPs and starch/PVA nanoscaffolds with embedded CuO NPs (Fig. 3A–B), using a Bruker D8 Advance diffractometer ($\text{CuK}\alpha$ source, $\lambda = 1.5406\text{ \AA}$, 40 kV , 40 mA , $2\theta: 5\text{--}90^\circ$), highlighted the crystalline properties. CuO NPs showed distinct diffraction peaks at 32.75° , 35.70° , 38.05° , 48.66° , 57.94° , 61.33° , and 75.48° 2θ , corresponding to d-spacings of $1.59\text{--}3.67\text{ \AA}$. These matched the monoclinic phase of CuO (JCPDS card 48-1548), affirming purity and the absence of secondary phases such as Cu_2O . The strongest peak at 35.70° (attributed to the $(002)/(111)$ planes) had a net area of $141.86\text{ Cps} \times ^\circ$. Scherrer's equation, based on peak broadening (FWHM: $0.1^\circ\text{--}0.9^\circ$), estimated crystallite sizes between $20\text{--}50\text{ nm}$. The nanoscaffolds displayed broader peaks at 21.35° , 22.97° , and 43.14°

2 θ (d-spacing: 2.09–4.16 Å), reflective of semi-crystalline starch/PVA matrices. CuO peaks were still visible at 35.70°, 57.28°, and 61.33° 2 θ , although with lower relative intensity (~15.9%) compared to the pure NPs, confirming successful embedding. The broad hump between 10–30° indicated a largely amorphous structure, while reduced CuO crystallinity was inferred from broader FWHM values (0.16°–1.06°). A negative intensity anomaly at 39.46° 2 θ was likely due to instrumental noise or measurement error. Overall, the results verified the synthesis of monoclinic CuO NPs and their effective inclusion within the polymeric scaffold, whose amorphous character supports particle stabilization.

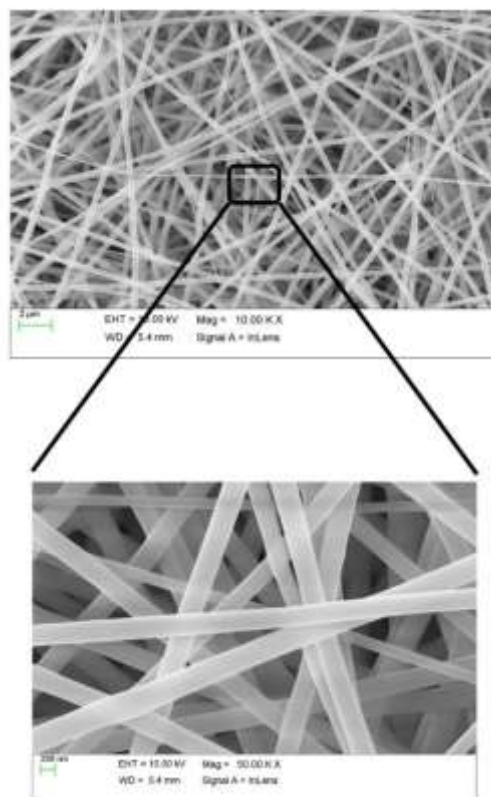


Fig. 4. SEM analysis of synthesized CuO NPs and CuO-loaded scaffolds.

3.4. SEM analysis

SEM analysis (Fig. 4) using JSM-IT800 NANO SEM (with gold sputtering) showed that the green-synthesized CuO NPs were spherical to irregular in shape, with sizes ranging from 50 to 200 nm. Some larger clusters were visible, attributed to limitations in surface charge leading to aggregation, corroborating DLS data. The NP surfaces appeared rough, likely due to phytochemical capping during green synthesis. SEM images of starch/PVA nanoscaffolds embedded with CuO NPs revealed uniform, bead-free electrospun fibers with average diameters between 150–300 nm and a porous architecture conducive to biomedical applications. Bright protrusions below 200 nm seen on the fiber surfaces indicated even distribution of CuO NPs, confirming successful loading without compromising the structural framework of the scaffolds.

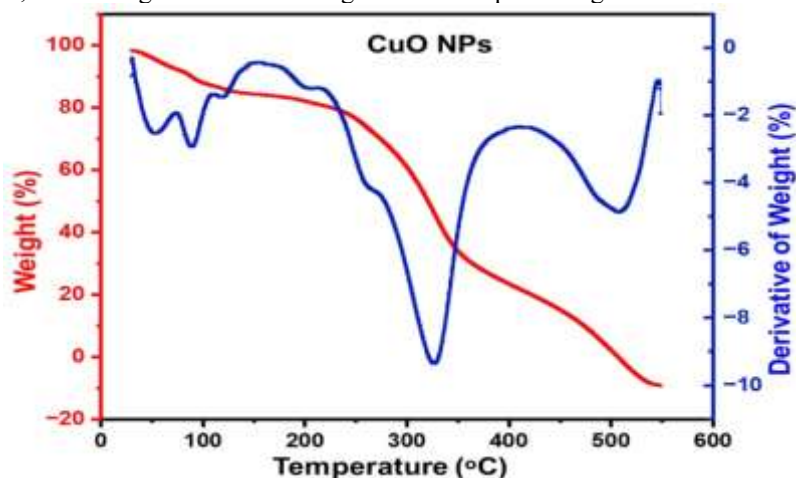


Fig. 5. Thermal degradation behavior of CuO-incorporated nanoscaffolds.

3.5. TGA

Thermal analysis (Fig. 5) of starch/PVA nanoscaffolds containing CuO NPs (sample mass: 2.335 mg), performed under nitrogen flow using a PerkinElmer TGA 8000 (heating rate: 15°C/min, range: 30–550°C), demonstrated a total mass loss of 35.87%, leaving 64.13% residue attributed to CuO content and thermally stable components. Degradation occurred in three phases: (1) Water evaporation (~50–90°C, peak at 53.71°C, rate: -2.54%/min), accounting for ~2–3% weight

reduction; (2) Major decomposition ($\sim 262.78^{\circ}\text{C}$ onset, peak at 326.36°C , rate: $-2.93\%/ \text{min}$), indicating starch/PVA polymer breakdown via C–C and C–O bond cleavage; and (3) Final decay ending around 363.12°C , linked to char residue degradation. The embedded CuO NPs improved thermal stability, raising the degradation threshold compared to native starch/PVA ($<250^{\circ}\text{C}$), as NP presence limits polymer chain motion. Residual weight ($\sim 64.13\%$) closely aligns with the estimated CuO NP content (30–40%), validating their stable incorporation and suggesting suitability of the scaffold for thermally demanding biomedical environments like sterilization.

3.6. Zeta Potential and DLS

DLS measurements (Fig. 6) of CuO NPs dispersed in water at 25°C yielded a hydrodynamic Z-average diameter of 125.6 nm (Fig. 6) and a polydispersity index (PI) of 0.475, indicating moderate polydispersity. Zeta potential (Fig. 7) assessed by Electrophoretic Light Scattering showed a surface charge of -10.03 mV and conductivity of 0.1813 mS/cm , reflecting moderate dispersion stability, although it falls short of the $\pm 30 \text{ mV}$ standard for high stability. The negative surface charge implies inter-particle electrostatic repulsion, helping reduce aggregation. Analysis conditions included a dispersant viscosity of 0.887 cP and dielectric constant of 78.5. Count rates were 1400 kcps (DLS) and 3720 kcps (ELS), and the quality factor of 1.754 with phase plot stability confirmed reliable data. These outcomes suggest a need for surface modifications to improve stability and lower polydispersity for enhanced biomedical compatibility.

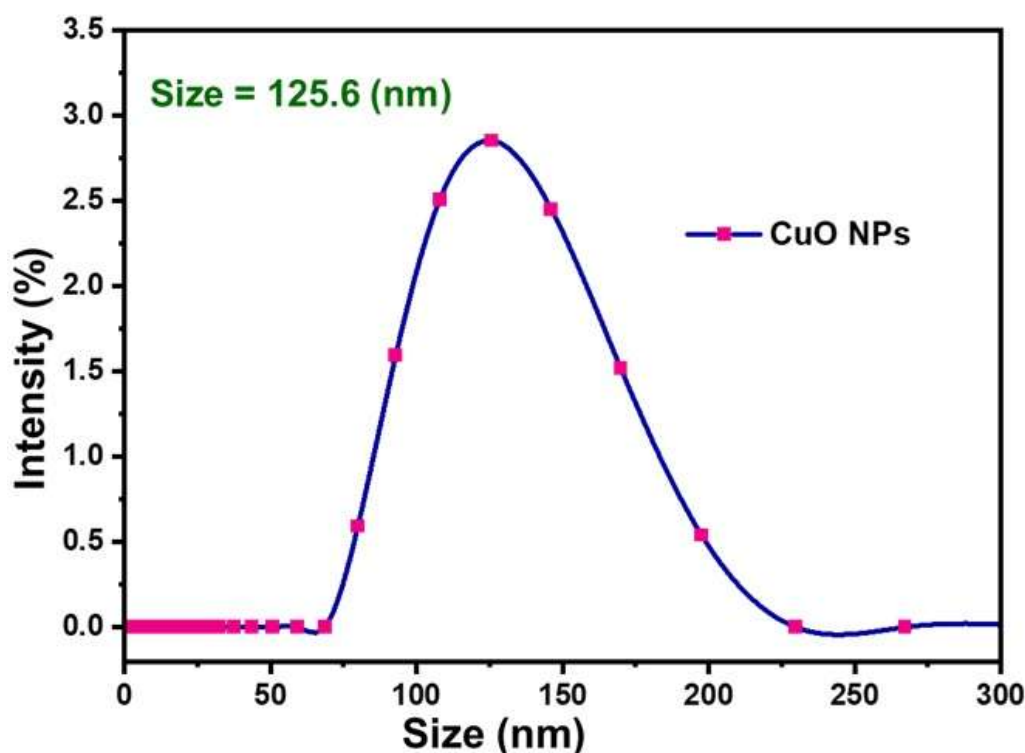


Figure 6: DLS particle size analysis of CuO NPs

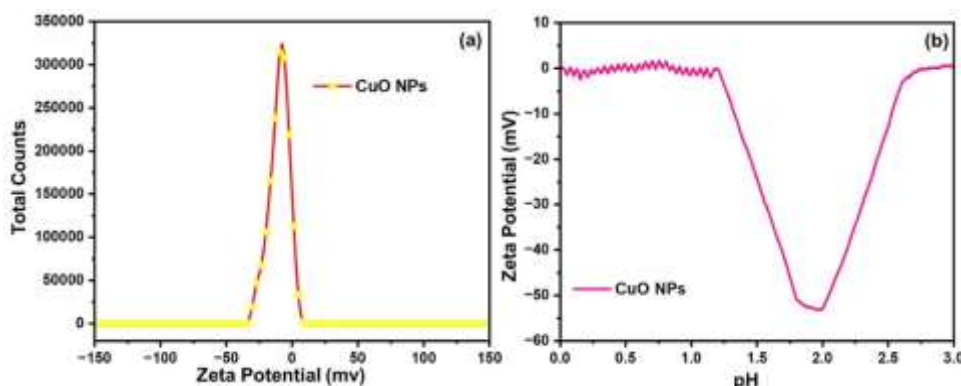


Fig.7. Zeta-potential analysis of CuO NPs

3.7. Antimicrobial potential

The antimicrobial activity of *P. variegata* extract ($50 \mu\text{g/mL}$), CuO NPs ($50 \mu\text{g/mL}$), and starch/PVA electrospun nanoscaffolds ($50 \mu\text{g/mL}$) was tested against bacterial and fungal pathogens using the agar well diffusion assay (Table 1). Against *Escherichia coli*, the algal extract showed an inhibition zone of $16 \pm 0.83 \text{ mm}$, while CuO NPs and nanoscaffolds exhibited zones of $23 \pm 0.58 \text{ mm}$ and $30 \pm 0.16 \text{ mm}$, respectively. For *Klebsiella pneumoniae*, the extract, CuO NPs, and nanoscaffolds produced zones of $15 \pm 0.27 \text{ mm}$, $20 \pm 0.14 \text{ mm}$, and $28 \pm 0.18 \text{ mm}$. *Staphylococcus aureus* was inhibited by zones of $14 \pm 0.49 \text{ mm}$ (extract), $18 \pm 0.32 \text{ mm}$ (CuO NPs), and $25 \pm 0.33 \text{ mm}$ (nanoscaffolds). Similarly, *Citrobacter koseri* displayed zones of $15 \pm 0.52 \text{ mm}$ (extract), $19 \pm 0.47 \text{ mm}$ (CuO NPs), and $26 \pm 0.46 \text{ mm}$ (nanoscaffolds). For fungal

strains, *Aspergillus niger* showed inhibition zones of 13 ± 0.43 mm (extract), 16 ± 0.65 mm (CuO NPs), and 23 ± 0.62 mm (nanoscaffolds), while *Penicillium* species exhibited zones of 14 ± 0.65 mm (extract), 18 ± 0.39 mm (CuO NPs), and 25 ± 0.35 mm (nanoscaffolds). The positive controls, ciprofloxacin (bacteria) and voriconazole (fungi), demonstrated higher efficacy with zones ranging from 26 ± 0.65 mm to 33 ± 0.58 mm. All tests were conducted in triplicate, with larger zones indicating stronger antimicrobial activity.

Table 1: Antimicrobial activity of *Pocockiella variegata* extract, CuO NPs, and starch/PVA electrospun nanoscaffolds at concentration of 50 µg/mL against various pathogenic organisms

		Ciprofloxacin (Antibiotic)	Voriconazole (Antifungal)	Algal Extract	CuO NPs	Nano- scaffolds
	<i>Escherichia coli</i>	33 ± 0.58	-	16 ± 0.83	23 ± 0.58	30 ± 0.16
	<i>Klebsiella pneumoniae</i>	30 ± 0.14	-	15 ± 0.27	20 ± 0.14	28 ± 0.18
	<i>Staphylococcus aureus</i>	28 ± 0.32	-	14 ± 0.49	18 ± 0.32	25 ± 0.33
	<i>Citrobacter koseri</i>	29 ± 0.47	-	15 ± 0.52	19 ± 0.47	26 ± 0.46
	<i>Aspergillus niger</i>	-	26 ± 0.65	13 ± 0.43	16 ± 0.65	23 ± 0.62
	<i>Penicillium</i> species	-	28 ± 0.39	14 ± 0.65	18 ± 0.39	25 ± 0.35

4. DISCUSSION

The UV-Visible spectrum of the *P. variegata* extract exhibited a prominent peak at 267 nm, indicative of UV-absorbing compounds such as phenolic groups or conjugated aromatic systems, which are often associated with bioactive properties. The absence of significant absorption in the visible range (400–700 nm) suggests minimal chlorophyll content, likely due to degradation during extraction. In contrast, the CuO NPs displayed an atypical surface plasmon resonance (SPR) peak at 292 nm, deviating from the conventional range (570–600 nm). This shift may result from NP size effects, surface oxidation, or interactions with stabilizing agents. The sharp decline in absorbance post-peak suggests good monodispersity with minimal aggregation, which is favorable for antimicrobial applications [50-53].

FTIR analysis confirmed the presence of functional groups involved in NP stabilization and scaffold formation. The broad peak at 3219.71 cm^{-1} in CuO NPs corresponds to OH/N-H stretching, likely from biomolecules acting as capping agents. Peaks at 1509.94 cm^{-1} (aromatic stabilizers) and $672\text{--}463\text{ cm}^{-1}$ (Cu–O bonds) validate successful CuO NP synthesis. For starch/PVA nanoscaffolds, characteristic peaks at 3295.71 cm^{-1} (OH), 2923.65 cm^{-1} (C–H), and $1141\text{--}1088\text{ cm}^{-1}$ (C–O–C) confirm polymer integrity. The shift in OH stretching suggests hydrogen bonding between CuO NPs and the scaffold matrix, enhancing structural stability. The appearance of a C=O peak (1711.09 cm^{-1}) may indicate PVA oxidation or interactions with CuO NPs, which could influence scaffold durability and functionality [54-56].

XRD patterns confirmed the monoclinic phase of CuO NPs (JCPDS 48-1548), with prominent peaks at 32.75° , 35.70° , and 38.05° 2θ . The absence of Cu_2O peaks indicates high purity. Scherrer's analysis estimated crystallite sizes between 20–50 nm, consistent with SEM observations. The nanoscaffolds exhibited broader peaks (21.35° , 22.97° 2θ), reflecting their semi-crystalline nature, while retained CuO peaks (35.70° , 57.28° 2θ) confirmed NP incorporation. The reduced intensity ($\sim 15.9\%$) and increased peak broadening suggest partial amorphization due to polymer interactions, which may enhance NP dispersion and scaffold flexibility [57-61].

SEM images revealed spherical to irregular CuO NPs (50–200 nm) with some aggregation (500–1000 nm), likely due to surface charge limitations. The starch/PVA nanoscaffolds displayed uniform, bead-free fibers (150–300 nm diameter) with a porous structure, ideal for biomedical applications. Bright protrusions on fiber surfaces confirmed well-dispersed CuO NPs, ensuring effective antimicrobial functionality without compromising scaffold integrity [62, 63].

TGA demonstrated a three-stage degradation profile for CuO-loaded scaffolds, with a residual mass of 64.13%, aligning with expected CuO content (30–40%). The enhanced thermal stability (degradation onset at 262.78°C vs. $<250^\circ\text{C}$ for pure starch/PVA) suggests that CuO NPs restrict polymer chain mobility, improving scaffold suitability for high-temperature applications like sterilization [64, 65].

DLS revealed moderate polydispersity (PI: 0.475) with a dominant particle size of 881.5 nm, indicating some aggregation. The zeta potential (-10.03 mV) suggests limited colloidal stability, falling below the $\pm 30\text{ mV}$ threshold for long-term dispersion. Surface modifications (e.g., polymer coatings) could enhance stability for biomedical use [66-70].

The antimicrobial assays demonstrated dose-dependent activity, with nanoscaffolds exhibiting the highest inhibition (e.g., 30 ± 0.16 mm for *E. coli*), followed by CuO NPs (23 ± 0.58 mm) and algal extract (16 ± 0.83 mm). Similar trends were observed for other pathogens, suggesting synergistic effects between CuO NPs and the polymeric matrix. While ciprofloxacin and voriconazole showed superior activity (26–33 mm), the nanoscaffolds' performance highlights their potential as alternative antimicrobial materials, particularly for wound dressings or coatings [71-73].

The integrated characterization confirms successful CuO NP synthesis, effective embedding into starch/PVA scaffolds, and notable antimicrobial activity. Future work should focus on optimizing NP dispersion and scaffold stability for clinical applications.

5. CONCLUSION

This study successfully demonstrated the green synthesis of CuO NPs using *P. variegata* extract and their effective incorporation into starch/PVA electrospun nanoscaffolds for antimicrobial applications. Spectroscopic analyses (UV-Vis, FTIR, XRD) confirmed the formation of pure, monoclinic CuO NPs with bioactive phytochemical capping, while SEM revealed uniform nanofiber scaffolds with well-dispersed NPs. The nanoscaffolds exhibited enhanced thermal stability (TGA) and moderate colloidal stability (DLS), though further surface modifications could improve dispersion. Antimicrobial assays highlighted the superior efficacy of CuO-loaded scaffolds against bacterial (*E. coli*, *S. aureus*) and fungal (*A. niger*, *Penicillium*) pathogens, with inhibition zones comparable to or exceeding those of standalone CuO NPs and algal extract. The synergistic effects of CuO NPs and the polymeric matrix suggest promising potential for biomedical uses, such as wound dressings or antibacterial coatings. Future research should optimize NP loading and scaffold stability for clinical scalability, ensuring prolonged antimicrobial activity and biocompatibility. Overall, this work provides a sustainable, nanocomposite-based strategy to combat microbial resistance.

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Data Availability Statement: The full dataset of physiochemical characterization associated with this work is published in Mendeley Data [Dataset] Physiochemical characterization of fabricated starch-based nanoscaffolds incorporated with *Pocockiella variegata* extract-mediated green synthesised copper oxide nanoparticles (DOI: 10.17632/6tddv74p8k.1). Disclosure: The material characterization data and figures (e.g., UV, SEM, FTIR, XRD, TGA, Zeta Potential and particle sizer) presented here were generated from a common dataset and are being reused across multiple related studies on nanomaterials. All biological analyses and conclusions are original to each manuscript.

Disclosure: The material characterization data and figures (e.g., UV, SEM, FTIR, XRD, TGA, Zeta Potential and particle sizer) presented here were generated from a common dataset and are being reused across multiple related studies on nanomaterials. All biological and environmental analyses and conclusions are original to each manuscript.

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