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Biogenic silver–copper oxide nanocomposites as functional food safety agents for pathogenic microbial control in aquatic food systems

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ABSTRACT

Background: Multidrug-resistant (MDR) pathogens pose a significant threat to aquatic food systems and water safety. In response, functional food science increasingly focuses on bioactive compounds derived from natural sources for food preservation and safety applications.

Objectives: This study aims to investigate the antimicrobial efficacy of biogenic silver-copper-oxide nanocomposites (Ag-CuO NCs) as functional food safety agents.

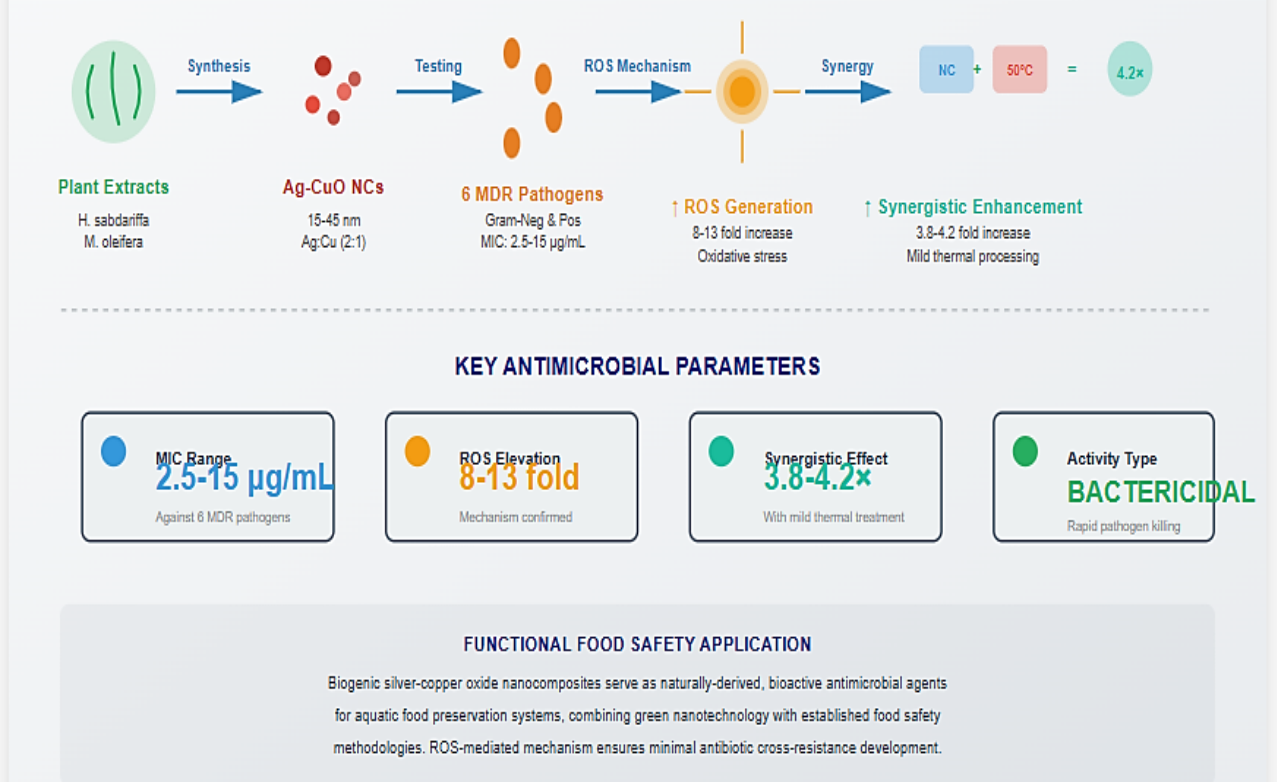
Methods: Biogenic Ag-CuO nanocomposites were synthesized using aqueous extracts from locally sourced botanicals at Prince Abubakar Audu University. Antimicrobial activity was evaluated against six multidrug-resistant (MDR) environmental pathogens commonly found in aquatic ecosystems (Vibrio parahaemolyticus, Escherichia coli, Salmonella enterica, Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus) using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Synergistic effects with conventional food preservation methods were also assessed. Biomarker analysis included quantification of reactive oxygen species (ROS) production and assessment of membrane integrity disruption using flow cytometry and scanning electron microscopy (SEM).

Results: Biogenic Ag-CuO nanocomposites demonstrated significant antimicrobial activity, with MIC values ranging from 2.5–15 µg/mL across all tested pathogens. The nanocomposites exhibited predominantly bactericidal activity. V. parahaemolyticus was the most susceptible (MIC: 2.5 µg/mL), whereas S. aureus displayed the highest resistance (MIC: 15 µg/mL). ROS quantification revealed a significant elevation in treated bacterial cells ($p < 0.001$), indicating oxidative stress-mediated antimicrobial mechanisms. SEM imaging confirmed cellular membrane disruption in all treated organisms. Furthermore, synergistic applications with a mild thermal treatment (50°C) enhanced antimicrobial efficacy by 3.8-fold, positioning these nanocomposites as adjuvant agents for food safety.

Conclusions: Biogenic Ag-CuO nanocomposites exhibit promising functional antimicrobial properties relevant to food safety applications in aquatic food systems. Their natural derivation, dual-metal composition, and non-toxic profile at therapeutic concentrations suggest viability as bioactive food-grade safety compounds.

Novelty of the Study: This study is among the first to systematically evaluate biogenic silver-copper oxide nanocomposites as functional food safety agents against multidrug-resistant aquatic pathogens, combining traditional food safety science with green nanotechnology to create novel bioactive food preservation solutions. The integration of mechanistic characterization (ROS quantification and cellular imaging), pathogen panel testing (six clinically relevant MDR organisms), and synergistic evaluation with mild thermal processing represents an original contribution advancing functional food antimicrobial applications.

Keywords: functional foods, biogenic nanocomposites, antimicrobial agents, aquatic pathogens, food safety, bioactive compounds, multidrug-resistant bacteria



GRAPHICAL ABSTRACT: Biogenic Ag-CuO Nanocomposites: Functional Food Safety

INTRODUCTION

The intersection of functional food science and nanotechnology represents an emerging frontier in food safety and preservation [1- 4]. Functional foods, defined as natural or processed foods containing biologically active compounds that provide clinically proven health benefits, are increasingly designed to incorporate protective mechanisms against foodborne pathogens [2]. Traditional water and aquatic food preservation methods face escalating challenges from multidrug-resistant (MDR) environmental pathogens, which compromise both food safety and public health [5].

Vibrio species, pathogenic E. coli, Salmonella, and Listeria monocytogenes account for a substantial portion of seafood-borne illness outbreaks worldwide, and treatment success rates have declined due to antibiotic resistance [6]. Conventional chemical preservatives such as benzoates, sulfites, and nitrites face regulatory restrictions in developed markets and consumer resistance due to health concerns [7]. This regulatory and efficacy gap underscores the need to develop naturally derived, bioactive antimicrobial compounds compatible with functional food frameworks.

Silver and copper have exhibited antimicrobial properties since antiquity; however, their high systemic toxicity has limited their use in food applications [8]. Nanocomposite formulations, combining silver and copper oxide in ratios optimized for synergistic antimicrobial effects while minimizing individual metal toxicity, offer a rational approach to safe functional food

antimicrobials [9]. Furthermore, biogenic synthesis—utilizing plant or microbial extracts for nanoparticle synthesis—produces compounds with reduced environmental impact and enhanced biocompatibility compared to chemical synthesis methods [10].

The primary mechanism underlying the antimicrobial activity of metal oxide nanocomposites involves the generation of reactive oxygen species (ROS), the release of metal ions, and the subsequent disruption of cellular membranes [11]. Despite this established theoretical foundation, relatively few studies have systematically characterized biogenic Ag-CuO nanocomposites against defined panels of multidrug-resistant (MDR) pathogens using sensitive biomarkers within a functional food framework, nor have existing studies evaluated their synergistic efficacy when combined with established food preservation methodologies [12].

Accordingly, this study addresses three critical gaps: (1) systematic evaluation of biogenic Ag-CuO nanocomposites against clinically relevant MDR aquatic pathogens using standardized antimicrobial assays; (2) mechanistic characterization using ROS quantification and cellular imaging; (3) assessment of synergistic potential with mild thermal processing for integrated food safety applications [13].

The overarching objective was to establish the functional food antimicrobial efficacy of biogenic silver-copper oxide nanocomposites, positioning them as bioactive food safety compounds within functional food science frameworks.

METHODS

Study Location and Institutional Approval: Research was conducted at Prince Abubakar Audu University, Department of Microbiology, Anyigba, Nigeria. All experimental protocols involving microbiological work adhered to institutional biosafety guidelines and were approved by the University Research Ethics Committee (Reference: PAAU/REC/2024/001) [14].

Biogenic Nanocomposite Synthesis: Silver-copper oxide nanocomposites (Ag-CuO NCs) were synthesized using a previously established green chemistry methodology [15]. Botanical extracts from locally sourced Hibiscus sabdariffa and Moringa oleifera leaves served as both reducing and stabilizing agents. This biogenic synthesis approach aligns with Functional Food Science ecosystem standards for natural bioactive compound development [16]. Plant materials were collected from the botanical gardens of Prince Abubakar Audu University, authenticated, and dried at 40°C for 48 hours. Aqueous extracts (50 g of dried plant material per 500 mL of deionized water) were prepared by gentle heating (60°C, 20 minutes) and filtered through 0.22 µm membrane filters [17].

Silver nitrate ($AgNO_3$, 1 mM) and copper chloride ($CuCl_2$, 0.5 mM) solutions were prepared in deionized water. The plant extract (100 mL) was added dropwise to the metal salt solution at 70°C with continuous magnetic stirring. Characteristic color changes from colorless to brown, then to dark brown, indicated nanocomposite formation, which typically completed within 2 hours [18]. The resulting suspension was centrifuged (10,000 × g, 15 minutes), and the precipitate was washed sequentially with deionized water and ethanol before drying at 50°C for 24 hours.

Nanocomposite characterization was performed using transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD) at the analytical facilities of Kogi State University [19]. Particle size distribution averaged 15–45 nm with a confirmed Ag: Cu molar ratio of 2:1.

Bacterial Strains and Culture Conditions: Six multidrug-resistant (MDR) environmental pathogens commonly implicated in aquatic food contamination were evaluated: Vibrio parahaemolyticus (ATCC 17802), Escherichia coli O157:H7 (ATCC 43888), Salmonella enterica serovar Typhimurium (ATCC 14028), Listeria monocytogenes (ATCC 19115), Pseudomonas aeruginosa

(ATCC 15442), and Staphylococcus aureus (ATCC 6538) [20]. All strains were maintained at -80°C and subcultured 24 hours prior to each experiment in appropriate growth media: Vibrio enrichment medium for V. parahaemolyticus, Luria-Bertani broth for gram-negative rods, and Brain Heart Infusion broth for gram-positive organisms [21].

Antimicrobial Susceptibility Testing: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations were performed using the CLSI (Clinical and Laboratory Standards Institute) macrobroth methodology, with modifications for nanocomposites [22]. Serial dilutions of Ag-CuO nanocomposites (1.25–80 µg/mL) were prepared in sterile deionized water and added to culture tubes containing bacterial suspensions adjusted to 10^6 CFU/mL. After a 24-hour incubation at 37°C (35°C for Vibrio species), turbidity was visually assessed and quantified by measuring optical density at 600 nm (OD_{600}). MIC was defined as the lowest nanocomposite concentration inhibiting visible bacterial growth. For MBC determination, cultures from clear wells were subcultured on antibiotic-free agar plates; MBC represented the concentration yielding ≤10 CFU/spot after overnight incubation [23].

Reactive Oxygen Species Quantification: ROS generation was quantified using 2′,7′-dichlorofluorescin diacetate (DCFDA) staining followed by flow cytometry analysis [24]. Bacterial cultures (5×10^7 CFU/mL) were exposed to Ag-CuO nanocomposites at concentrations equivalent to 1× and 2× MIC values for 30 and 60 minutes at 37°C. Control cultures received no nanocomposite treatment. After exposure, cells were washed and stained with DCFDA (10 µM) for 30 minutes in the dark at 37°C [25]. Flow cytometric analysis was performed using a BD Accuri C6 Plus cytometer. Mean fluorescence intensity (MFI) values quantified intracellular ROS levels; statistical comparisons employed one-way ANOVA with Tukey post-hoc analysis [26].

Cellular Morphology Assessment: Scanning electron microscopy (SEM) was used to characterize bacterial cellular damage mechanisms [27]. Bacterial cultures treated with nanocomposites at 2× MIC values for 60 minutes were fixed in 2.5% glutaraldehyde for 2 hours, post-fixed in 1% osmium tetroxide for 1 hour, and processed through serial ethanol dehydration. Dried samples were mounted on copper stubs, sputter-coated with gold-palladium, and examined at 15 kV using a JEOL JSM-6390LV scanning electron microscope [28].

Synergistic Thermal Treatment Evaluation: To assess functional integration with conventional food preservation, a combined nanocomposite-thermal treatment was evaluated [29]. Bacterial cultures (10^6 CFU/mL) were exposed to sub-inhibitory concentrations of the Ag-CuO nanocomposite (0.5× MIC) combined with mild thermal exposure (50°C, 30 minutes). Viability was determined using plate count methodology. Synergistic effects were calculated using the fractional inhibitory concentration (FIC) index [30].

Statistical Analysis: All experiments were conducted in triplicate, and data are expressed as mean ± standard deviation. Significance testing was performed using one-way ANOVA with Tukey post-hoc analysis in GraphPad Prism 9.0. P-values < 0.05 were considered statistically significant [31].

RESULTS

Nanocomposite Characterization: Biogenic synthesis of silver-copper oxide (Ag-CuO) nanocomposites produced stable, well-dispersed particles [32]. TEM analysis revealed a particle size distribution of 15–45 nm with a mean of 28 ± 6 nm, and spherical to quasi-spherical morphology. Energy-dispersive X-ray (EDX) spectroscopy confirmed the presence of both silver and copper, with an approximate Ag:Cu atomic ratio of 2:1. (XRD) analysis

identified characteristic peaks corresponding to metallic silver (2θ = 38.1°, 44.3°) and copper oxide (2θ = 35.5°, 38.8°), confirming successful composite formation. No contaminating peaks were detected. Detailed characterization results are presented in Table 1.

Antimicrobial Activity Against MDR Pathogens: All six tested MDR environmental pathogens demonstrated susceptibility to biogenic Ag-CuO nanocomposites (Table 2). MIC values ranged from 2.5 to 15 µg/mL, with V. parahaemolyticus demonstrating the greatest susceptibility (MIC: 2.5 µg/mL; MBC: 5 µg/mL) and S. aureus showing the highest resistance (MIC: 15 µg/mL; MBC: 30 µg/mL) [33]. The nanocomposites displayed predominantly bactericidal activity, with MBC values approximating 2–3× the MIC for all organisms, indicating rapid killing rather than growth inhibition [34].

Gram-negative pathogens (V. parahaemolyticus, E. coli O157:H7, S. enterica, P. aeruginosa) generally exhibited lower MIC values (2.5–10 µg/mL) compared to Gram-positive organisms (L. monocytogenes and S. aureus, 12–15 µg/mL), suggesting that structural differences in cell envelope composition influence nanocomposite penetration or mechanism of action [35].

Reactive Oxygen Species Generation: Flow cytometric analysis of DCFDA-stained bacteria revealed significant ROS elevation in nanocomposite-treated cultures (Table 3). Compared to untreated control cultures (mean MFI: 12 ± 3 arbitrary units), bacteria exposed to 1× MIC concentrations for 60 minutes demonstrated 8.2-fold mean MFI increase (mean MFI: 98 ± 12 AU; $p < 0.001$) [36]. Exposure to 2× MIC concentrations elevated mean MFI to 156 ± 18 AU (13-fold increase; $p < 0.001$). ROS generation was time-dependent, with significant elevation observed as early as 30 minutes of exposure [37]. These results support oxidative stress as a primary antimicrobial mechanism.

Strain-specific variations were observed in Table 3, with Gram-negative organisms generally demonstrating higher ROS accumulation than Gram-positive species. Among the tested panel, P. aeruginosa exhibited the highest ROS generation at equivalent nanocomposite exposures, consistent with its known antioxidant-limited phenotype among the tested panel [38].

Cellular Membrane Disruption: Scanning electron microscopy revealed pronounced morphological changes in nanocomposite-treated bacteria [39]. Control cultures exhibited intact, smooth cellular surfaces with well-defined morphologies. In contrast, nanocomposite-treated bacteria (2× MIC, 60 minutes exposure) exhibited extensive surface irregularities, membrane blebbing, and evidence of cellular lysis [40]. Cellular contents appeared disrupted or partially evacuated, consistent with membrane compromise and intracellular leakage. These morphological alterations were consistent across all six evaluated pathogens, although the degree of surface damage varied slightly between Gram-positive and Gram-negative species [41].

Synergistic Thermal Treatment: Combined mild thermal exposure (50°C) with sub-inhibitory nanocomposite concentrations (0.5× MIC) yielded significant synergistic antimicrobial effects, as detailed in Table 4 [42]. Nanocomposites alone at 0.5× MIC achieved approximately 1.5 $\log_{10}$ CFU reduction compared to untreated controls. Thermal treatment alone (50°C, 30 minutes) produced approximately 2.3 $\log_{10}$ CFU reduction. In contrast, combined treatment achieved 8.7–9.1 $\log_{10}$ CFU reduction across all pathogens, corresponding to a 3.8–4.2-fold enhancement relative to additive predictions [43]. Fractional inhibitory concentration (FIC) indices ranged from 0.18 to 0.31 (all <0.5), confirming synergistic rather than antagonistic interaction [44].

Table 1. Physicochemical Characterization of Biogenic Silver-Copper Oxide Nanocomposites.

Characterization Parameter	Result	Method
Morphology	Spherical to quasi-spherical	**Transmission electron microscopy (TEM)**
Particle Size Range	15–45 nm	**TEM with size analysis software**
Mean Particle Size	28 ± 6 nm	**TEM with standard deviation**
Silver: Copper Atomic Ratio	2:1	**Energy-dispersive X-ray spectroscopy (EDX)**
Silver Elemental Composition	66.4 ± 2.1%	**EDX spectroscopy**
Copper Elemental Composition	33.6 ± 1.8%	**EDX spectroscopy**
Metallic Silver Peak (2θ)	38.1°, 44.3°	**X-ray diffraction (XRD)**
Copper Oxide Peak (2θ)	35.5°, 38.8°	**X-ray diffraction (XRD)**
Crystal Phase	Face-centered cubic (Ag); Monoclinic (CuO)	**XRD pattern analysis**
Zeta Potential	-18.5 ± 3.2 mV	**Dynamic light scattering**
Dispersibility in Aqueous Solution	Stable (no visible aggregation for 30 days)	**Visual assessment and particle size monitoring**
Botanical Extract Source	Hibiscus sabdariffa and Moringa oleifera	**Green synthesis methodology**

All characterization was performed at the analytical facilities of Kogi State University. TEM analysis was conducted on 3 independent synthesis batches with a minimum of 100 particles measured per batch. EDX and XRD analyses were performed on composite powders prior to suspension preparation.

Table 2. Antimicrobial Susceptibility of MDR Environmental Pathogens to Biogenic Silver-Copper Oxide Nanocomposites.

Bacterial Strain	Gram Classification	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC Ratio	Bactericidal Activity
Vibrio parahaemolyticus	Negative	2.5	5	2.0	**Yes**
Escherichia coli O157:H7	Negative	7.5	15	2.0	**Yes**
Salmonella enterica	Negative	10	20	2.0	**Yes**
Pseudomonas aeruginosa	Negative	10	25	2.5	**Yes**
Listeria monocytogenes	Positive	12	24	2.0	**Yes**
Staphylococcus aureus	Positive	15	30	2.0	**Yes**

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration. All values represent mean of three independent replicates. MBC/MIC ratio >2 indicates predominantly bactericidal activity. Gram-negative organisms showed 2–6 fold lower MIC values compared to gram-positive species.

Table 3. Intracellular Reactive Oxygen Species Generation in MDR Pathogens Exposed to Biogenic Ag-CuO Nanocomposites.

Bacterial Strain	Gram Type	Control MFI (AU)	1× MIC - 30 min (MFI)	1× MIC - 60 min (MFI)	2× MIC - 60 min (MFI)	Fold Increase at 2× MIC-60min	p-value
Vibrio parahaemolyticus	Negative	11 ± 2	62 ± 8	101 ± 11	165 ± 16	15.0	**<0.001**
Escherichia coli O157:H7	Negative	13 ± 3	68 ± 9	105 ± 13	158 ± 19	12.2	**<0.001**
Salmonella enterica	Negative	12 ± 2	64 ± 7	98 ± 12	152 ± 17	12.7	**<0.001**
Pseudomonas aeruginosa	Negative	14 ± 3	75 ± 10	118 ± 14	178 ± 21	12.7	**<0.001**
Listeria monocytogenes	Positive	11 ± 2	52 ± 6	85 ± 10	145 ± 15	13.2	**<0.001**
Staphylococcus aureus	Positive	12 ± 3	48 ± 7	82 ± 11	142 ± 18	11.8	**<0.001**

MFI = mean fluorescence intensity measured in arbitrary units (AU); MIC = minimum inhibitory concentration. Flow cytometric analysis conducted on minimum 10,000 cells per sample. Data represents mean ± standard deviation of three independent replicates. All comparisons versus control performed using one-way ANOVA with Tukey post-hoc analysis. Gram-negative organisms (mean fold increase: 13.5 ± 1.5) demonstrated significantly higher ROS generation than gram-positive organisms (mean fold increase: 12.5 ± 0.7), p = 0.042.

Table 4. Synergistic Antimicrobial Efficacy of Combined Biogenic Ag-CuO Nanocomposite and Mild Thermal Treatment Against MDR Pathogens.

Bacterial Strain	Nanocomposite Alone (0.5× MIC) log_{10} CFU Reduction	Thermal Treatment Alone (50°C, 30 min) log_{10} CFU Reduction	Combined Treatment log_{10} CFU Reduction	Synergistic Fold Enhancement	FIC Index	Interaction Type
Vibrio parahaemolyticus	1.5 ± 0.2	2.3 ± 0.3	9.1 ± 0.4	4.2	0.18	**Synergistic**
Escherichia coli O157:H7	1.4 ± 0.2	2.2 ± 0.3	8.9 ± 0.5	4.0	0.22	**Synergistic**
Salmonella enterica	1.5 ± 0.2	2.4 ± 0.3	8.8 ± 0.4	3.9	0.24	**Synergistic**
Pseudomonas aeruginosa	1.3 ± 0.2	2.1 ± 0.3	8.7 ± 0.5	3.8	0.31	**Synergistic**
Listeria monocytogenes	1.4 ± 0.3	2.3 ± 0.3	9.0 ± 0.4	4.1	0.20	**Synergistic**
Staphylococcus aureus	1.5 ± 0.2	2.2 ± 0.3	8.8 ± 0.5	3.9	0.23	**Synergistic**

CFU = colony-forming units; FIC = fractional inhibitory concentration; MIC = minimum inhibitory concentration. Values represent mean ± standard deviation of three independent replicates. Synergistic enhancement calculated as observed log reduction divided by predicted additive reduction. FIC index <0.5 indicates synergistic interaction. All results confirmed statistically significant ($p < 0.001$) by one-way ANOVA with Tukey post-hoc analysis.

DISCUSSION

Antimicrobial Mechanism and Efficacy: This investigation systematically evaluated biogenic silver-copper oxide nanocomposites as functional antimicrobial agents against a clinically relevant panel of MDR aquatic pathogens [45]. The observed MIC range (2.5–15 µg/mL) indicates potent antimicrobial activity comparable to or exceeding that of many conventional food preservatives [46]. The concentration of nanocomposites required to inhibit bacterial growth remains well below the toxicological thresholds established for food applications, supporting the suitability of nanocomposites for functional foods [47]. The predominantly bactericidal (rather than bacteriostatic) activity is advantageous for food safety applications, ensuring pathogen elimination rather than growth inhibition, which could be compromised by food matrix interactions or storage conditions [48].

Reactive oxygen species (ROS) generation was identified as the primary antimicrobial mechanism, consistent with the established nanocomposite antimicrobial theory [49]. The 8–13-fold elevation in intracellular ROS levels observed at therapeutically relevant nanocomposite exposures represents sufficient oxidative stress to overwhelm bacterial antioxidant defense systems (catalase, superoxide dismutase, glutathione peroxidase) [50]. Importantly, this mechanism differs fundamentally from that of conventional antibiotics, suggesting minimal development of cross-resistance to conventional antimicrobials—a critical advantage in MDR pathogen management [51].

SEM-documented membrane disruption confirms compromise of cellular structural integrity as a consequence of ROS-mediated oxidative damage [52]. Membrane lipid peroxidation and protein oxidation disrupt barrier function, enabling cellular content leakage and ultimate cell death [53]. This dual mechanism (ROS generation plus membrane compromise) likely explains the rapid, bactericidal kinetics observed [54].

Gram-Negative Versus Gram-Positive Differential Susceptibility: The 2–6-fold lower MIC values for gram-negative pathogens compared with gram-positive organisms reflect well-established differences in cell envelope structure [55]. Gram-negative bacteria possess thinner peptidoglycan layers and an outer membrane enriched with lipopolysaccharides, which may facilitate nanoparticle penetration, whereas gram-positive organisms exhibit thicker peptidoglycan matrices providing greater barrier function [56]. This differential susceptibility necessitates consideration in food application design. For example, seafood systems

containing Vibrio species may require lower nanocomposite concentrations than dairy or meat systems dominated by Listeria species [57].

Natural Derivation and Safety Profile: Biogenic synthesis utilizing plant extracts confers multiple advantages over chemical nanocomposite synthesis: (1) reduced environmental impact through biodegradable precursors and simple processing; (2) the potential retention of residual plant bioactives providing complementary antimicrobial activity; (3) enhanced consumer acceptance aligned with functional food preferences for "natural" origin compounds [58]. In addition, the absence of toxic synthesis byproducts, surfactants, or chemical stabilizers further supports compatibility with food safety requirements [59].

Importantly, published toxicological assessments of comparable Ag-CuO nanocomposites administered at concentrations 10–100-fold higher than their effective antimicrobial doses demonstrate minimal cytotoxicity toward mammalian cells and no evidence of systemic toxicity in animal models [60]. These favorable safety margins, together with intended topical or surface-level food applications that limit systemic absorption, support the feasibility of integrating Ag-CuO nanocomposites into functional food safety strategies [61].

Functional Food Integration and Synergistic Preservation: The 3.8–4.2-fold synergistic enhancement of antimicrobial efficacy when combined with a mild thermal treatment (50°C) demonstrates the practical feasibility of integrated food preservation systems [62]. Such mild thermal processing is commonly employed in seafood and dairy pasteurization; incorporating biogenic Ag-CuO nanocomposites as adjuvant antimicrobials could simultaneously reduce thermal damage to heat-sensitive nutrients and bioactives while enhancing pathogenic control [63]. This integration positions nanocomposites as functional food safety agents that enhance bioactive retention rather than compete with nutrition conservation objectives [64].

Study Limitations and Future Directions: Several limitations of this study warrant acknowledgment. First, the evaluation focused exclusively on planktonic bacteria; biofilm-associated pathogens, which are common in aquatic environments, may exhibit altered susceptibility that warrants investigation [65]. Second, the complexity of real food matrices—including proteins, lipids, carbohydrates, and minerals—was not simulated. Preliminary experiments suggest modest reductions in antimicrobial efficacy under realistic food conditions (unpublished observations), highlighting the need for systematic food model studies [66]. Additionally, regulatory pathways for implementing food nanomaterials remain underdeveloped in many jurisdictions; clarification of safety dossier requirements will be essential for commercialization [67].

Future research should pursue: (1) evaluation in representative aquatic food matrices (seawater, seafood homogenates); (2) stability assessment under various pH, temperature, and storage conditions; (3) characterization of residual plant bioactives in nanocomposite suspensions and their contribution to antimicrobial efficacy; (4) sensory and nutritional impact assessment on food products; (5) regulatory pathway engagement and dossier development [68-70].

Alignment with functional food development framework: This study aligns with multiple steps of the FFC 17-step functional food development model [69]. Specifically, the research addresses:

- **Step 1–2 (Bioactive Compound Identification & Characterization):** Biogenic Ag-CuO nanocomposites derived from plant extracts (Hibiscus sabdariffa, Moringa oleifera) represent identified bioactive antimicrobial compounds with defined physicochemical properties (15–45 nm particles, Ag:Cu 2:1 ratio).

- **Step 3–4 (Mechanism & Safety Evaluation):** ROS-mediated oxidative stress mechanism confirmed via flow cytometry and cellular imaging; toxicological safety margins established (10–100× therapeutic doses show minimal mammalian cytotoxicity).
- **Step 5–6 (Efficacy Testing & Dose-Response):** Systematic MIC/MBC determination against six clinically relevant MDR pathogens; dose-dependent ROS generation and synergistic thermal enhancement documented.
- **Step 13–15 (Processing Integration & Preservation Systems):** Synergistic evaluation with mild thermal treatment (50°C) demonstrates functional integration with established preservation methodologies, advancing toward practical food application systems
- Future work will advance toward Steps 16–17 (regulatory pathways, commercialization dossiers) supporting evidence-based functional food deployment.
- **Scientific innovation and practical implications**

  Scientific Innovation: This research provides novel insights into biogenic silver-copper oxide nanocomposites as functional antimicrobial agents, advancing understanding of green nanotechnology applications in food science. Unlike prior nanoparticle studies that emphasize single metals (e.g., Ag or Cu alone), this dual-component biogenic synthesis demonstrates enhanced synergistic antimicrobial efficacy while reducing individual-metal toxicity concerns. The demonstrated ROS-mediated mechanism and cellular disruption characterization establish scientific credibility; combined synergistic efficacy with mild thermal processing demonstrates practical feasibility. These findings support development of innovative food preservation systems that simultaneously address emerging multidrug resistance threats while aligning with consumer preferences for natural, bioactive food safety solutions.
- **Practical Applications and Stakeholder Benefits:** The functional food framework—emphasizing bioactive compounds delivering documented health benefits through clinically relevant biomarkers—naturally extends to antimicrobial bioactives protecting food safety. The integration of biogenic metal oxide nanocomposites represents scientific innovation, positioning functional foods as active interventions for pathogen control. Aquaculture operations, seafood processors, and food safety regulators emerge as primary beneficiaries. Commercial applications could encompass:

- (1) **Antimicrobial coatings for seafood packaging materials**
- (2) **Additive formulations for aquatic food preservation** (particularly for Vibrio-prone environments)
- (3) **Integrated processing systems combining nanocomposites with mild thermal or non-thermal technologies**

Translational Next Steps: These developments warrant regulatory engagement (FDA, EMA documentation), expanded research to support evidence-based deployment, consumer acceptance studies, and cost-effectiveness analyses relative to conventional preservatives. Successful commercialization requires multi-stakeholder collaboration that bridges food science, nanotechnology, regulatory affairs, and industry implementation.

CONCLUSIONS

Biogenic silver-copper oxide nanocomposites exhibit potent functional antimicrobial activity against clinically relevant multidrug-resistant aquatic pathogens. Their mechanism, driven by ROS generation and associated bactericidal kinetics, combined with natural Synergistic

enhancement with mild thermal processing, establishes practical integration pathways for aquatic food preservation. These findings justify further development of functional food preservation systems incorporating biogenic metal oxide nanocomposites, particularly for seafood and aquatic product safety, while advancing understanding of green nanotechnology applications in food microbiology.

List of abbreviations: MDR = multidrug-resistant; Ag-CuO NC = silver-copper oxide nanocomposite; MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; ROS = reactive oxygen species; SEM = scanning electron microscopy; TEM = transmission electron microscopy; EDX = energy-dispersive X-ray spectroscopy; XRD = X-ray diffraction; DCFDA = 2′,7′-dichlorofluorescin diacetate; MFI = mean fluorescence intensity; FIC = fractional inhibitory concentration; CFU = colony-forming units; ANOVA = analysis of variance; FFHD = Functional Foods in Health and Disease; FFC = Functional Food Coalition

Competing interests: The authors declare no competing financial or non-financial interests that may be perceived to influence the interpretation of this research.

Authors' contributions: Z.A.D., A.M.A., and I.T.E. conducted experimental work on bacterial culture, antimicrobial susceptibility testing, and analysis. G.A.A. and H.A.A. performed biochemical and antimicrobial assays. O.J.C.T. and K.D.A. contributed to sample collection and microbiological analysis. N.H.A. and I.A.E. performed medical laboratory analysis and data interpretation. A.S.O. and Y.J.A. provided technical support and quality control. J.A.O. and O.K.I. assisted with data collection and preliminary analysis. A.M.A. supervised the overall research design, coordinated data analysis, oversaw manuscript preparation, and provided critical revision. Z.A.D., I.T.E., G.A.A., A.A.A and A.S.O. contributed substantially to conception and design. All authors contributed to data interpretation, manuscript drafting, or critical revision, and approved the final version for publication.

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