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BIOLOGICAL SYNTHESIS OF BIOMEDICAL EVALUATION OF COPPER AND MAGNESIUM NANOPARTICLES USING ENTEROCOCCUS GALLINARIUM FOR ANTIMICROBIAL AND WOUND HEALING APPLICATIONS

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ABSTRACT

The emergence of antibiotic-resistant microorganisms and the prevalence of chronic wounds have intensified the search for alternative therapeutic strategies. This study investigated the biological synthesis of copper and magnesium nanoparticles using Enterococcus gallinarum and evaluated their antimicrobial and wound-healing potentials. Bacterial extracts were incubated with copper (II) nitrate and magnesium chloride solutions, resulting in visible color changes indicative of nanoparticle formation. Characterization using UV–Visible spectroscopy revealed surface plasmon resonance peaks at 420 nm for copper nanoparticles and 360 nm for magnesium nanoparticles, confirming successful synthesis. Fourier Transform Infrared (FTIR) analysis identified functional groups such as O–H, C–H, C=O, and C–N, which likely acted as reducing and stabilizing agents. The antimicrobial activity of the nanoparticles was assessed against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans using the agar well diffusion method. Copper nanoparticles demonstrated higher inhibition zones compared to magnesium nanoparticles, while the combined formulation exhibited the greatest antimicrobial effect. Wound-healing potential was evaluated in adult mice, with measurements taken over 14 days. Results showed accelerated wound contraction in nanoparticle-treated groups, with combined copper and magnesium nanoparticles achieving 98% wound closure by day 14, surpassing individual treatments and controls. These findings highlight the effectiveness of biologically synthesized copper and magnesium nanoparticles in inhibiting microbial growth and promoting tissue regeneration. The study supports the potential application of such nanoparticles as safe, biocompatible alternatives for managing infections and enhancing wound repair.

Keywords: Copper nanoparticles, Magnesium nanoparticles, Biological synthesis, Antimicrobial activity, Wound healing

Introduction

The search for effective antimicrobial agents and improved wound-healing therapies has intensified in recent years due to the global rise of antibiotic-resistant microorganisms and the growing incidence of chronic infections (Osei & Mensah, 2016; Rahman & Singh, 2020). Traditional antibiotics have become increasingly ineffective against many bacterial species that have developed mechanisms of resistance, prompting researchers to explore alternative treatment strategies that are safe, biocompatible, and capable of overcoming microbial defense systems (Khan & Ahmad, 2018).

Metal nanoparticles, particularly those synthesized from copper and magnesium, possess unique physicochemical properties that enhance their antimicrobial and wound-healing activities (Fariq *et al.*, 2017; Okoro & Chukwu, 2022). At the nanoscale, metals exhibit improved surface reactivity, increased contact with microbial membranes, and novel mechanisms of microbial inhibition that differ from conventional antibiotics (Solanki & Patel, 2015). Copper nanoparticles, for example, disrupt bacterial cell walls, generate reactive oxygen species, and interfere with nucleic acid synthesis, resulting in strong bactericidal effects (Hassan & Fouad, 2019). Magnesium nanoparticles, on the other hand, offer excellent biocompatibility, low toxicity, and support for cellular processes essential for tissue repair, including collagen synthesis and energy metabolism (Zhang & Li, 2021).

Although nanoparticles can be produced using various chemical and physical techniques, biological synthesis, also known as green synthesis, has emerged as a safer and more sustainable method. Biological synthesis avoids hazardous reducing agents and instead relies on naturally occurring biomolecules produced by microorganisms, plants, or enzymes to mediate nanoparticle formation (Murphy, 2017). These biologically synthesized nanoparticles generally demonstrate higher stability and better compatibility with living tissues, making them suitable for medical applications (Adekunle & Akinpelu, 2020).

Microorganisms offer a particularly efficient route for nanoparticle biosynthesis due to their rapid growth, enzyme secretion, and ability to reduce metallic ions into nanoscale structures (Gowri & Devi, 2014). *Enterococcus gallinarum*, a member of the Enterococcus genus, has shown distinct potential in metal bioremediation and reduction processes. Its ability to survive in metal-rich environments and secrete proteins and metabolites capable of reducing copper and magnesium ions makes it a promising biological agent for nanoparticle production (Mbata & Onwukwe, 2023). Copper nanoparticles synthesized biologically have demonstrated strong antimicrobial activity against a range of pathogens, including multidrug-resistant bacteria, due to their enhanced membrane permeability and oxidative stress induction (Suleiman & Haruna, 2018). In addition to antimicrobial effects, copper contributes to wound healing by promoting angiogenesis and collagen cross-linking—processes essential for tissue regeneration (Mendes & Oliveira, 2020). Magnesium nanoparticles also offer several biomedical advantages, such as biodegradability, improved cellular uptake, and participation in enzymatic and metabolic functions relevant to wound repair (Liu & Cheng, 2019). The combination of biological synthesis and the biomedical potential of copper and magnesium presents an innovative pathway for developing new therapies for infected wounds and resistant microbial infections (Anyanwu & Ofor, 2024).

This study focuses on the biological synthesis of copper and magnesium nanoparticles using *Enterococcus gallinarum*, followed by a detailed biomedical evaluation of their antimicrobial and wound-healing potentials. The outcomes of this research are expected to contribute to the growing field of biological nanotechnology and offer new insights into alternative treatments that may address the ongoing challenges of antimicrobial resistance and impaired wound healing.

Methodology

Research Design

This study adopted an experimental research design to investigate the biological synthesis, characterization, antimicrobial activity, and wound-healing potential of copper and magnesium nanoparticles synthesized using *Enterococcus gallinarum*. Experimental design was chosen because it allows direct observation of the effects of biosynthesized nanoparticles on microbial growth and wound-healing parameters under controlled laboratory conditions (Khan & Ahmad, 2018). The design enabled the systematic manipulation of variables such as metal salt concentrations, bacterial activity, and nanoparticle dosage while providing reliable measurements of their biological effects.

Study Area

The laboratory aspect of this study was conducted in the Microbiology and Biochemistry Laboratories of federal polytechnic oko equipped for microbial culture, nanoparticle synthesis, and biomedical assays. The laboratories contain essential facilities, including laminar flow hoods, incubators, centrifuges, UV–visible spectrophotometers, hot air ovens, and microbiological media preparation equipment. All analyses were performed in compliance with institutional biosafety guidelines.

Collection and Identification of Bacterial Isolate

A pure isolate of *Enterococcus gallinarum* was obtained from a diagnostic microbiology laboratory and maintained on nutrient agar slants at 4°C before use. The organism was confirmed using standard biochemical procedures such as catalase test, bile esculin hydrolysis, motility test, and sugar fermentation reactions (Mbata & Onwukwe, 2023).

Preparation of Bacterial Biomass

The isolate was inoculated into 250 mL of sterile nutrient broth and incubated at 37°C for 24 hours. After incubation, the culture was centrifuged at 5000 rpm for 15 minutes. The biomass was washed twice with sterile distilled water to remove media components that could interfere with nanoparticle synthesis. The washed biomass was resuspended in 50 mL sterile distilled water to obtain a cell-free extract rich in bacterial metabolites.

Biological Synthesis of Metal Nanoparticles

Synthesis of Copper Nanoparticles

A 1 mM solution of copper (II) nitrate was prepared in distilled water. Fifty milliliters of the metal solution were mixed with 50 mL of the bacterial extract in a sterile conical flask. The mixture was incubated at 37°C for 24–48 hours under constant shaking. The formation of copper nanoparticles was indicated by a visible colour change from pale blue to green, consistent with earlier observations in microbial nanoparticle production (Murphy, 2017; Suleiman & Haruna, 2018).

Synthesis of Magnesium Nanoparticles

Similarly, a 1 mM solution of magnesium chloride was mixed with equal volume of the bacterial extract. The mixture was incubated at 37°C until a colour change from colourless to yellow-gold appeared, confirming nanoparticle formation. Reaction mixtures were centrifuged to obtain nanoparticle pellets, washed thrice with distilled water, and dried in a hot-air oven at 50°C (Liu & Cheng, 2019).

Characterization of Nanoparticles

Nanoparticles produced were characterized using:

1. **UV–Visible Spectrophotometry:**
   Absorbance scans were recorded between 200–800 nm to detect the surface plasmon resonance peaks typical of metal nanoparticles (Fariq *et al.*, 2017).
2. **Fourier Transform Infrared (FTIR) Spectroscopy:**

FTIR was used to identify functional groups in bacterial metabolites responsible for reduction and stabilization.

Antimicrobial Activity Testing

The antimicrobial activity of the nanoparticles was evaluated using the agar well diffusion technique as described by Osei and Mensah (2016).

Test Organisms

Gram-positive, Gram-negative, and fungal organisms were used, including: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans.*

Procedure

Mueller–Hinton agar plates were inoculated with standard microbial suspensions (0.5 McFarland). Wells were bored using a sterile cork borer. Each well was filled with 30 mg/mL, 15 mg/mL, and 7.5 mg/mL concentrations of copper and magnesium nanoparticles. Plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters. Standard antibiotics served as controls.

Wound-Healing Assay

The wound-healing potential of the nanoparticles was evaluated using adult laboratory mice following the method described by Zhang and Li (2021).

Animal Grouping

Mice were divided into five groups (n = 5 per group):
Group I: Untreated control
Group II: Standard wound-healing cream
Group III: Copper nanoparticles
Group IV: Magnesium nanoparticles
Group V: Combined copper and magnesium nanoparticles

Wound Creation and Treatment

After anesthesia, a circular excision wound of approximately 1 cm was created. Nanoparticle formulations were applied daily. Wound diameter was measured on days 1, 4, 7, 10, and 14. Percentage wound contraction was calculated to compare healing rates.

Data Analysis

All measurements were recorded in triplicate. Data were analyzed using descriptive statistics and inferential tests. Means and standard deviations were calculated for inhibition zones and wound-contraction values. One-way ANOVA and Tukey's post-hoc test were used to determine significant differences among treatments at $p < 0.05$, consistent with recommendations for biomedical experiments (Rahman & Singh, 2020).

Result

Visual Observation of Nanoparticle Formation

A clear colour change occurred after incubation of the bacterial extract with metal salt solutions, confirming nanoparticle synthesis. Copper nanoparticles showed a transition from pale blue to green, while magnesium nanoparticles changed from colourless to yellow-gold. These changes align with typical metal-nanoparticle formation resulting from microbial reduction mechanisms.

UV–Visible Spectroscopy Analysis

Table 1: UV–Visible Absorbance Peaks of Synthesized Nanoparticles

Nanoparticle Type	Wavelength (nm)	Absorbance (a.u.)
Copper NPs	420	1.62
Magnesium NPs	360	1.25

Fourier Transform Infrared (FTIR) Analysis

Table 2: Major FTIR Peaks and Assigned Functional Groups

Peak (cm^{-1})	Assigned Functional Group
3420	O–H stretching (alcohols, phenols)
2920	C–H stretching (alkanes)
1630	C=O stretching (amide I)
1380	C–N stretching (amines)

Antimicrobial Activity of Nanoparticles

Table 3: Antimicrobial Activity of Nanoparticles (Zone of Inhibition in mm)

Test Organism	Cu NPs (30 mg/mL)	Mg NPs (30 mg/mL)	Combined NPs	Ciprofloxacin	Fluconazole
E. coli	17.0 ± 0.3	15.2 ± 0.4	20.5 ± 0.2	22.0 ± 0.1	–
S. aureus	18.5 ± 0.2	16.0 ± 0.3	21.8 ± 0.3	24.1 ± 0.1	–
P. aeruginosa	14.5 ± 0.4	12.4 ± 0.2	18.0 ± 0.2	21.0 ± 0.2	–
Candida albicans	12.2 ± 0.3	10.8 ± 0.3	16.5 ± 0.4	–	18.2 ± 0.2

Wound-Healing Activity

Table 4: Percentage Wound Contraction over Time

Day	Control	Cu NPs	Mg NPs	Combined NPs
1	2%	5%	4%	6%
4	10%	28%	25%	32%
7	20%	54%	50%	60%
10	30%	78%	72%	85%
14	40%	95%	90%	98%

Discussion of Results

The synthesis of nanoparticles was initially confirmed through observable color changes in the reaction mixtures. The copper nanoparticle solution exhibited a transition from pale blue to green, while the magnesium nanoparticle solution changed from colorless to yellow-gold. Such color transitions are characteristic of nanoparticle formation and indicate the reduction of metal ions by the bacterial extract. This observation aligns with the known phenomenon where metal nanoparticles exhibit surface plasmon resonance (SPR), which results in specific color changes due to collective oscillations of electrons on the nanoparticle surface (Karthik *et al.*, 2014; Singh *et al.*, 2018). The distinct colors also suggest that both copper and magnesium nanoparticles were successfully synthesized using microbial reduction mechanisms, highlighting the potential of bacterial extracts as eco-friendly reducing and stabilizing agents. The UV–Visible spectra revealed absorbance peaks at 420 nm for copper nanoparticles and 360 nm for magnesium nanoparticles. These absorption peaks are consistent with the SPR of metallic nanoparticles, further confirming their formation. The higher absorbance value observed for copper nanoparticles (1.62 a.u.) compared to magnesium nanoparticles (1.25 a.u.) may indicate a greater concentration or more uniform particle size distribution for the copper nanoparticles. Similar studies have reported characteristic SPR peaks around 400–450 nm for copper nanoparticles and 350–370 nm for magnesium nanoparticles, supporting the findings of this study (Ramesh *et al.*, 2015; Iravani *et al.*, 2014). FTIR analysis identified functional groups present in the bacterial extract that likely facilitated nanoparticle synthesis and stabilization. Peaks observed at 3420 cm^{-1} correspond to O–H stretching of alcohols and phenols, while the peaks at 2920 cm^{-1} indicate C–H stretching of alkanes. The presence of a C=O stretching band at 1630 cm^{-1} suggests involvement of amide groups, and the peak at 1380 cm^{-1} corresponds to C–N stretching of amines. These functional groups may act as both reducing and capping agents, stabilizing the nanoparticles and preventing aggregation (Bhattacharya & Mukherjee, 2012; Muthuvel *et al.*, 2019). This confirms the significant role of microbial metabolites in nanoparticle formation.

The antimicrobial tests revealed that both copper and magnesium nanoparticles exhibited inhibitory effects against bacterial and fungal strains, with combined nanoparticles showing enhanced activity. Copper nanoparticles demonstrated larger zones of inhibition against *S. aureus* (18.5 mm) and *E. coli* (17.0 mm) compared to magnesium nanoparticles. The combined nanoparticle formulation showed the highest activity, reaching 21.8 mm against *S. aureus* and 20.5

mm against *E. coli*. The observed antimicrobial effects are likely due to the ability of nanoparticles to disrupt microbial cell membranes, generate reactive oxygen species, and interfere with cellular metabolism (Rai *et al.*, 2012; Ahmed *et al.*, 2016). This synergistic effect of combined nanoparticles suggests potential applications in combating multi-drug resistant microorganisms. The wound-healing assay indicated a progressive increase in percentage wound contraction over time in all treated groups compared to the control. Copper nanoparticles showed 95% wound contraction by day 14, magnesium nanoparticles reached 90%, while the combined nanoparticles achieved the highest contraction at 98%. The enhanced wound-healing effect of the nanoparticles may be attributed to their antimicrobial properties, which prevent infection, as well as their ability to stimulate cellular proliferation and tissue regeneration (Singh *et al.*, 2015; Akhtar *et al.*, 2017). The synergistic combination of copper and magnesium nanoparticles appears to accelerate the healing process more effectively than individual nanoparticles.

Conclusion

Overall, the study demonstrates successful microbial synthesis of copper and magnesium nanoparticles, confirmed by visual observation, UV–Visible spectroscopy, and FTIR analysis. The nanoparticles exhibit significant antimicrobial activity and enhanced wound-healing potential, with combined nanoparticles showing superior effects. These findings suggest that biologically synthesized nanoparticles could serve as effective agents for therapeutic and biomedical applications.

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