ORIGINAL ARTICLE

Determination the Antimicrobial Activity of Copper Oxide Synthesized by Extracellular *Lactobacillus Plantarum* against some Multidrug Resistant Bacteria Isolated from Urinary Tract Infection

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ABSTRACT

Background: *Copper oxide nanoparticles (CuO NPs) have attracted increasing attention for their versatile applications in catalysis, biosensing, anticancer therapy, and other biomedical fields. Green synthesis using microbial extracellular components offers a sustainable and eco-friendly alternative to conventional chemical methods.* ***Objective:*** *This study aimed to elucidate the biosynthetic process of copper oxide nanoparticles ($Cu(NO_3)_2$ NPs) utilizing extracellular components from the environmental isolate Lactobacillus plantarum as a natural reducing and stabilizing agent.* ***Methodology:*** *For nanoparticle synthesis, 1 g of copper nitrate was added to 10 mL of extracellular filtrate. The obtained CuO NPs were characterized using Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), and Field Emission Scanning Electron Microscopy (FE-SEM). Antibiotic resistance profiles of the bacterial isolates and the antibacterial activity of the CuO NPs were assessed.* ***Results:*** *FE-SEM analysis revealed spherical CuO nanoparticles forming nano-cluster aggregates. All isolates exhibited 100 % resistance to cefotaxime and 91 % resistance to ceftriaxone and lincomycin, while resistance to other antibiotics ranged from 18 % to 73 %. Multidrug resistance was observed in all isolates, with at least four antibiotics resisted. The biosynthesized CuO NPs showed strong antibacterial activity, producing inhibition zones of 33 mm against Staphylococcus saprophyticus (150 % concentration) and 19 mm against Burkholderia cepacia (100 % concentration).* ***Conclusion:*** *Lactobacillus plantarum proved effective for the eco-friendly biosynthesis of CuO NPs. Given the high prevalence of antibiotic resistance, these nanoparticles demonstrate promising potential as alternative antimicrobial agents against multidrug-resistant pathogens.*

INTRODUCTION

L. plantarum is a member of the group of lactobacilli that are capable of heterofermentation. The species is adaptable and hardy, and it can be found in a wide variety of biological niches. These niches include dairy, meat, fish, cheese, and a variety of plant or vegetable fermentations. In addition, there is evidence that certain strains of *L. plantarum* have successfully colonized the intestines of mammals, including humans[1]. It is possible to differentiate *Lactobacillus plantarum* from other types of bacteria due to the characteristics that these bacteria possess[2]. Not only are these components utilized as reducing agents, but they are also utilized as biological defenses against a variety of stressors, including phage infections, toxic metallic ions, and dehydration. On the other hand, iron particles that are smaller than one micron in size are referred to as nanoscale iron particles. Because they have such a large surface area, they are more reactive than that of other substances[3]. In the presence of oxygen and water, they promptly undergo oxidation, which results in the production of free iron ions. It has also been explored whether or not they are useful in restoring industrial sites that have been contaminated by chlorinated organic compounds. They are used frequently in medical and laboratory settings [4]. Urinary tract infections (UTIs) are the most common bacterial infection worldwide and are defined as the presence of at least one of the following urinary tract symptoms: fever (greater than 38°C) in patients aged 65 or younger, lower back pain, urinary frequency, dysuria or retention, pain with urination, bloody or burning urination, nausea, and vomiting [5].

They affect both sexes and all ages, with females being more susceptible than males due to anatomical

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and physiological differences in the urogenital system, as well as lifestyle differences. The incidence of UTIs is higher in elderly men. When infection occurs, different parts of the urinary tract may be affected. Bacteria are widely distributed in various environments, including soil, salt and fresh water, plants, and animals, and they also infect humans. This widespread distribution indicates their high adaptability to different environments. Therefore, bacteria are among the most widespread, complex, and ecologically diverse organisms [6].

The advent of multidrug-resistant bacterial strains has significantly increased the frequency of UTI, rendering this condition a major social and economic burden globally[5]. By 2016, an estimated 150 million cases occurred annually worldwide[7]. Hospital infections are one of the most important and dangerous health problems facing the world, as they increase the mortality rate and complications of the disease, as well as the patient's length of stay in the hospital [8]. What increases the complexity of this problem is that many bacterial species have become resistant to commonly used antibiotics, as they can survive and multiply in the presence of the antibiotic [9]. The ability of bacteria to acquire resistance to antibiotics is a result of the development of several mechanisms that enable them to resist, such as the occurrence of genetic mutations or the acquisition of resistance plasmids. Despite the increasing knowledge of bacterial pathogenesis and the application of modern treatments, the rate of bacterial infections remains high[10]. Therefore, there was a need to find modern methods and strategies and to use new antibacterial agents, including the use of nano-inorganic metal oxides. This study aimed to elucidate the biosynthetic process of copper oxide nanoparticles ($Cu(NO_3)_2$ NPs) utilizing extracellular components from the environmental isolate *Lactobacillus plantarum* as a natural reducing and stabilizing agent.

METHODOLOGY

Isolation and identification of pathogenic bacteria

Pathogenic bacteria were collected from patients at Tikrit Teaching Hospital in Tikrit, Iraq, who were suffering from UTIs. Following the culture of the samples on mannitol salt agar, blood agar, and MacConkey agar, the samples were then incubated at 37 degrees Celsius for twenty-four hours. Initial identification of the bacteria was accomplished by analyzing the morphological characteristics of the colonies, and subsequent examination of the bacteria under a microscope was performed following Gram staining. A large number of biochemical assays were carried out in order to identify the bacteria that were isolated[11], and the identification was validated by utilizing a VITEK 2 Compact System.

Antibiotic sensitivity test

The CLSI 2024 guidelines [12] were followed by all isolates. The Kirby-Bauer disk diffusion method [13] was used to test the antibacterial susceptibility of Mueller-Hinton agar. The antimicrobial drugs that were tested at different doses are listed in Table 1. A digital caliper was used to measure the diameters of the inhibition zone surrounding the disks. According to the CLSI 2024 recommendations, the outcome was classified as sensitive, moderate, or resistant.

Synthesis of copper oxide nanoparticles

***Lactobacillus plantarum* isolate**

Species were obtained by collecting samples of Lactobacillus species that were isolated from a food product that had been fermented. MRS broth was used to culture the isolates, and the directions provided by the manufacturer were followed [14]. The VITEK2 system was utilized in order to identify all of the bacterial species that were isolated through the utilization of conventional biochemical assays and identification procedures.

Extracellular production

Following the incubation of *Lactobacillus plantarum* isolates in MRS broth at a temperature of 28 °C for a period of 48 hours, the extracellular solution was collected by centrifugation at 8000 revolutions per minute for a period of ten minutes [15].

Synthesis of copper oxide nanoparticles from extracellular

Utilizing $Cu(NO_3)_2$ (Indian) with modification[16], a biological method approach was employed to synthesize copper oxide nanoparticles (CuO NPs) from intracellular sources for Lactobacillus spp. During a standard operation: 5. gm In order to dissolve $Cu(NO_3)_2$, fifty milliliters of the extracellular product solution of Lactobacillus SPP was utilized. After that, the solution was dispersed in an ultrasonication bath for ten minutes in order to improve the mixing of the components. Finally, the mixture was shaken and incubated in the dark for a whole night. Following the centrifugation of the resulting solution at 8000 revolutions per minute for ten minutes, the solution was subsequently washed twice with deionized distilled water in order to remove any remaining extracellular cells. After that, the substance was dried in an oven at a temperature of 40 °C for an entire night, which resulted in the production of a brown powder. This powder was then kept in a dark place for use in the future.

Characterisation of Nanoparticles

Atomic force microscope (AFM)

After applying fifty microliters of the CuO nanoparticle solution that was created, the slide was then dried in an oven at a temperature of 40°C for an entire night. An atomic force microscope (Phywe measure nano/ England) was utilized in order to investigate the surface topography of nanoparticles and to ascertain the diameters of these particles. At the

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University of Baghdad's College of Sciences, the Department of Chemistry and Biotechnology Department Laboratory, the material was examined according to the established protocols.

Fourier transmission infrared spectroscopy (FTIR)

Subsequently, the material was compacted into a thin pellet after being mixed with KBr. The Fourier transform infrared spectrometer (FTIR) in Germany was used to gather infrared spectra over the entire wavelength range of 400-4000 cm-1. For the purpose of demonstrating the functional group of the CuO nanoparticles, this assay was utilized [17,18]. Within the College of Sciences at the University of Baghdad, the Department of Chemistry and Biotechnology Laboratory was responsible for conducting the analysis on the sample.

Scanning electron microscopic analysis (SEM)

The generated nanoparticles were coated with gold and subjected to scanning electron microscopy (SEM) imaging (Angstrom/Advanced/USA) in order to examine the form and surface topology of the nanoparticles. Following the removal of any extra powder and the creation of thin films of the sample, the material was next analyzed using a SEM. A laboratory at the Chemistry and Biotechnology Department of the College of Sciences at Baghdad University was responsible for conducting the analysis on the sample[19].

Inhibitory activity of CuO NPs:

To test the inhibitory activity of synthesized CuO NPs against 11 multidrug-resistant Gram-negative and Gram-positive bacteria isolated from patients with UTI infections, 0.2 ml of fresh cultures of each organism were inoculated into 5 ml of sterile nutrient broth (Himedia/India) and incubated for 3–5 h to standardize the culture to McFarland standards (106 CFC). Three copies of each microbe were created by spreading 100 µl of revived culture on Mueller Hinton Agar media (Himedia/India) using a spreader. Following gel puncture (6 mm), 0.1 ml of different CuO synthesis dilutions from extracellular sources were added to wells. (75, 100, and 150%) were loaded in a well, along with a solvent control sample of deionized water. The plates were incubated overnight at 37°C. The inhibitory zone was measured in mm. The post-incubation inhibitory zone around the well was assessed [18,20]. After three runs, averages and SD were calculated.

RESULTS

Isolation and identification

The results indicated that 11 samples exhibiting positive bacterial growth were obtained from UTIs. The isolates were identified by microscopic morphological criteria and several biochemical tests for both gram-positive and gram-negative bacteria. The identification was validated via the VITEK 2 Compact system. The identification revealed that there were 5 isolates of gram-positive bacteria, constituting 45.5%, while gram-negative bacteria included 6 isolates, accounting for 54.5%, as presented in Table 1.

Table 1: Results of the biochemical and morphological properties of isolated microorganisms

Isolated bacteria	Gram stain	IND	VP	MR	C	Oxidase	Catalase	Coagulase
Escherichia coli	G - ve	+	-	+	-	-	+	ND
Proteus mirabilis	G - ve	-	V	+	V	-	+	ND
Klebsiella pneumoniae	G - ve	-	+	V	+	-	+	ND
Citrobacter koseri	G - ve	V	-	+	+	-	+	ND
Enterobacter aerogenes	G - ve	-	+	-	+	-	+	ND
Burkhoderia.cepacia	G - ve	ND	ND	ND	ND	+	+	ND
Streptococcus.agalactia	G+ ve	ND	ND	ND	ND	-	-	ND
Staphylococcus.aureus	G+ ve	ND	ND	ND	ND	-	+	+
Staphylococcus.waxenri	G+ ve	ND	ND	ND	ND	-	+	-
Staphylococcus.hominis	G+ ve	ND	ND	ND	ND	-	+	-
Staphylococcus.saprophyticus	G+ ve	ND	ND	ND	ND	-	+	-

G^- ve: Gram negatine bacteria, G^+ ve: Gram positive bacteria, IND: indole, MR:methyl red, VP: Voges proskaur, C: Citrate utilization, ND: not don

Sensitivity of bacterial isolates to antibiotics:

The effectiveness of ten different medications was evaluated against twelve different bacterial species that were isolated from UTI. It was established by the findings presented in Table 2 that all isolates exhibited resistance to the majority of the antibiotics that were utilized in this investigation. All isolates showed the highest percentage of resistance, 100%, against cefotaxime, while the resistance to ceftriaxone and lincomycin decreased slightly by 91%. Thus, their resistance to other types of antibiotics ranged from 18% to 73%. All isolates showed multiple resistance to eleven different types of antibiotics, with a resistance rate of no less than four antibiotics.

Table 2: Antimicrobial sensitivity of bacterial isolates against antibiotics

Antibiotics / Bacteria species	ATM	N	CN	IPM	AMC	L	CTX	MEM	OFX	CRO
Klebsiella pneumonia	S	S	R	S	S	R	R	S	S	R
Citrobacter.koseri	S	R	S	S	R	R	R	S	S	R
Streptococcus.agalactia	R	R	S	S	R	R	R	S	S	S
Staphylococcus.waxenri	R	S	S	S	R	R	R	S	S	R
Staphylococcus.hominis	R	R	S	S	R	R	R	R	S	R
Staphylococcus.saprophyticus	R	R	S	S	R	S	R	S	S	R
Burkhoderia.cepacia	R	R	S	S	R	R	R	S	R	R
Escherichia.coli	R	S	R	S	S	R	R	S	R	R
Enterobacter.aerogenes	R	S	S	R	S	R	R	R	R	R
Staphylococcus.aureus	R	R	S	R	R	S	R	S	S	R
Proteus.mirabilis	S	R	S	S	R	R	R	R	S	R

ATM: Aztreonam, N: Neomycin, CN: Gentamycin, IPM: Imipenem, AMC: Amoxicillin/ Clavulanic acid, L: Lincomycin, CTX: Cefotaxime, MEM: Meropenem, OFX: Ofloxacin, CRO: Ceftriaxon,

4- Characterization of biological synthesis CuONPs

AFM analysis of CuO from intracellular:

Figure 1 AFM images indicate that the biosynthesized CuO nanoparticles are spherical in shape. The average diameter determined by AFM was 44.85 nm (Figure 1).

FE-SEM study of CuO derived from extracellular sources

FE-SEM was utilized to capture images of the sample at a magnification of 50,000x. Concentrated on (Figure 2). The entire sample exhibits soft planes and a consistent morphology, characterized by CuO nanocluster centers ranging from 34.6 to 67.62 nm.

By analyzing the CuO nanoparticles using the extracellular FT-IR spectra, the functional groups contained inside them have been revealed by FT-IR research. Biologically produced nanoparticles' FT-IR absorption spectra are shown in Figure 3.

A pronounced peak in the FTIR spectra for CuO at 459.03 - 414.07 cm^{-1} was seen, ascribed to the OH stretching mode. The notable peak at 1629.74 cm-1 signified the presence of crystallographic H2O molecules, particularly the O–H bending vibration.

The prominent peaks at 455.17 cm^{-1} and 572.82 cm^{-1} corresponded to the Cu–O band[15].

The inhibitory activity of CuO NPs test:

Copper Oxide NPs inhibitory activity was investigated against 11 species of Gram-negative and Gram-positive bacteria isolated from patients with UTI by the use of the agar well diffusion technique[18,20]. The outcomes of CuO NPs from extracellular inhibitory activity are illustrated in Figure 4. The inhibitory activity was directly correlated with the concentration of CuO NPs.

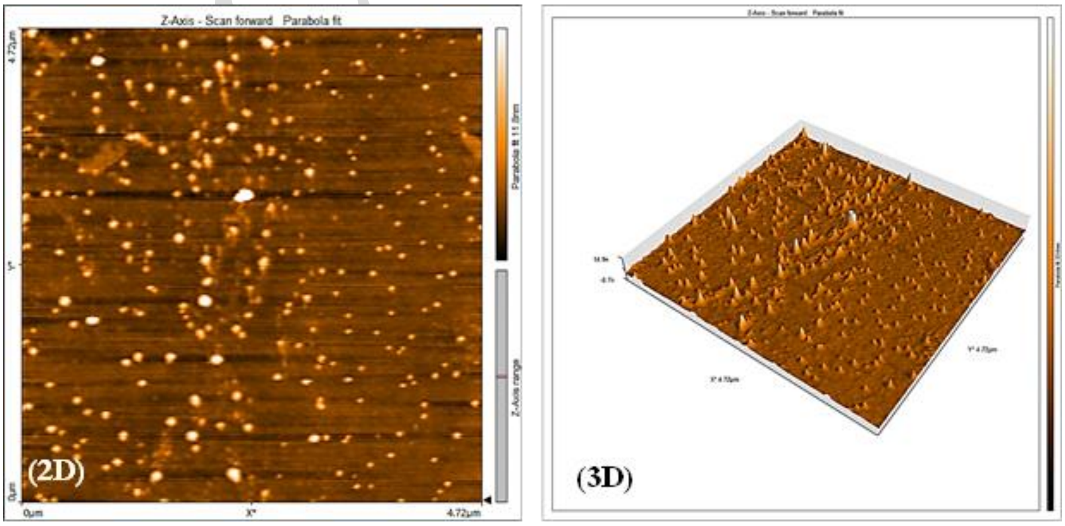

Fig. 1: AFM of CuO nanoparticles produced via extracellular methods demonstrates 2D and 3D topological characteristics.

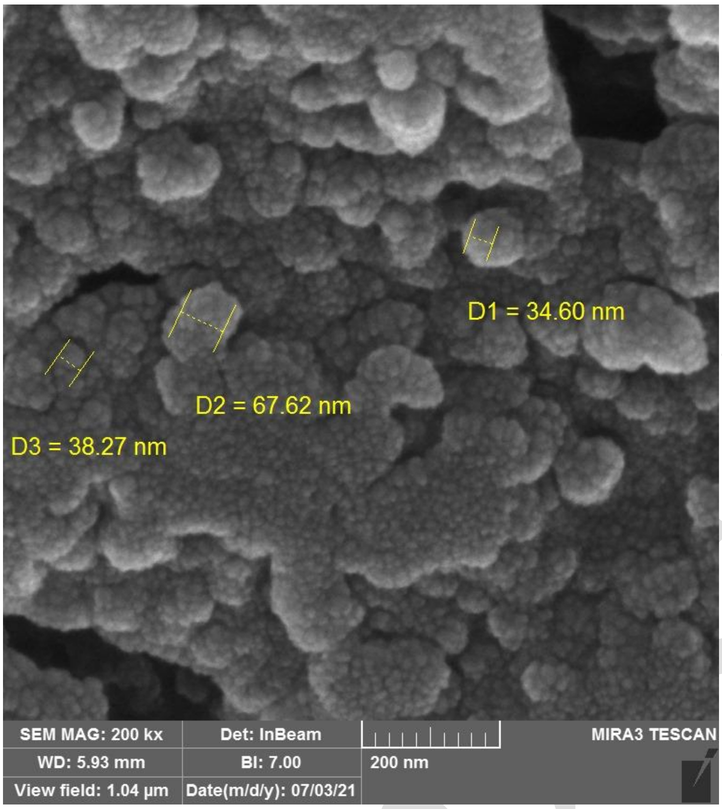

Fig. 2: FE-SEM Images of CuO NPs synthesized using Extracellular

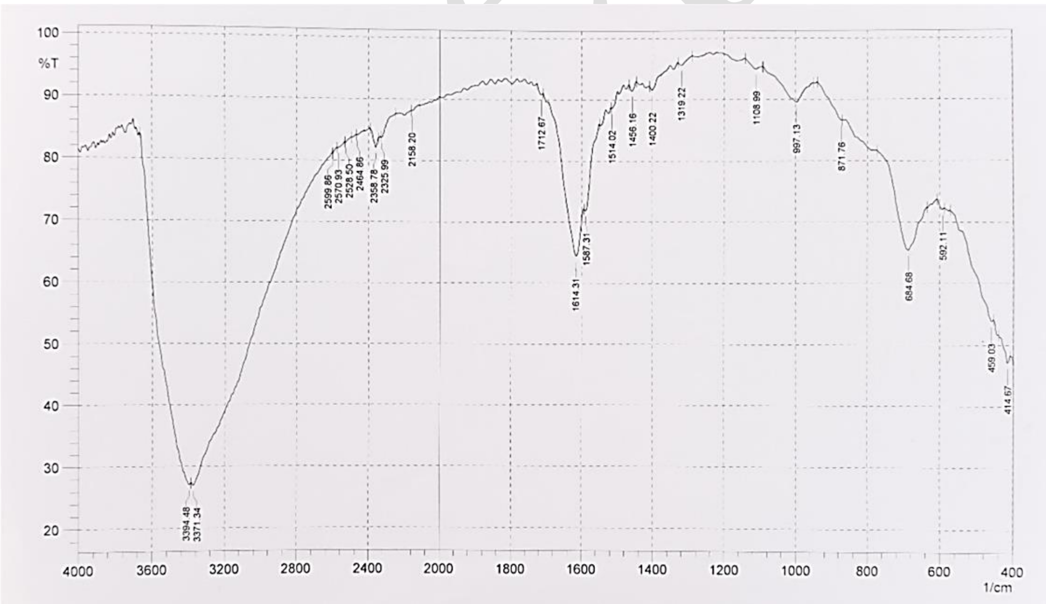

Fig. 3: FT-IR image of CuO NPs synthesized using extracellular

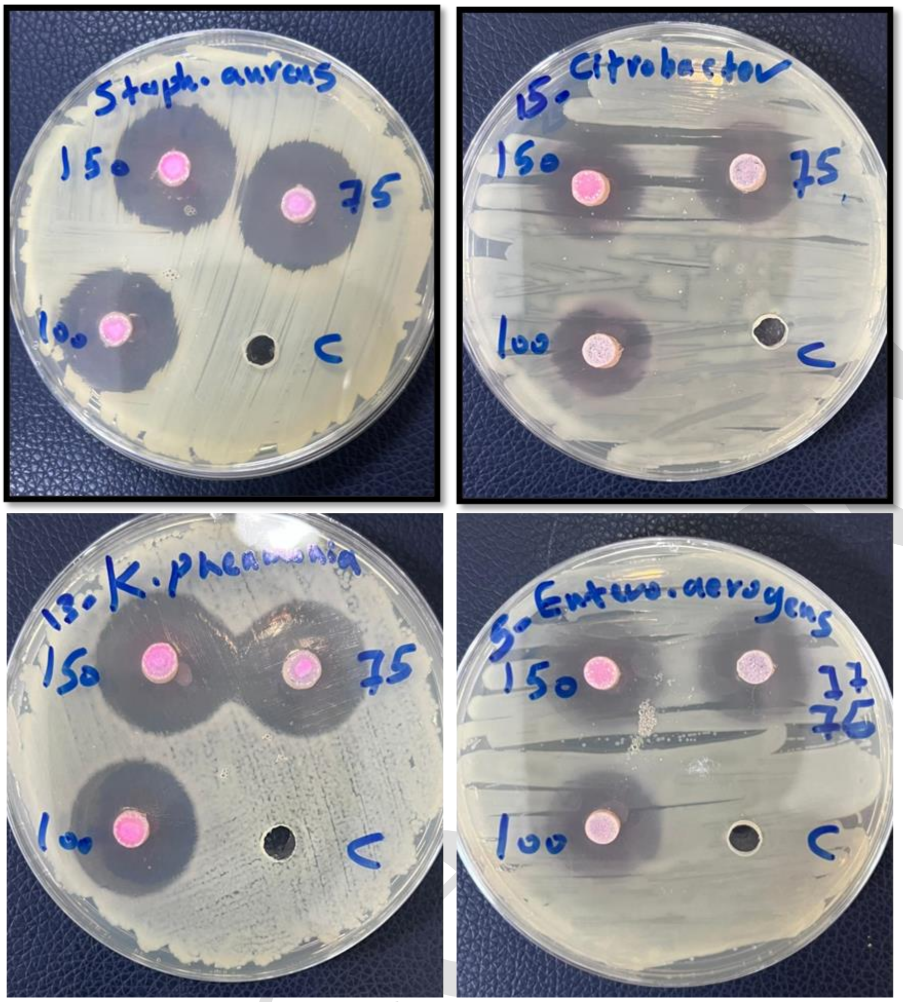

Fig. 4: Inhibitory activity of CuO NPs from extracellular against multi drug resistant isolated bacteria

Table (3) shows that the inhibition zone differed according to the concentration of CuO and species of isolated bacteria under study. The highest inhibition zone was reached at 30 mm at a concentration of 75% against *Staph. hominis* and *Staph. saprophyticus*, while the lowest inhibition zone was 20 mm against *Strept.agalactia* and *Burkhoderia cepacia.* Whereas the highest inhibition zone was located at 100% CuO NP concentrations and was 32 mm against *Staph.saprophyticus*, while the lowest inhibition zone was 19 mm against *Burkhoderia cepacia.* As for the highest concentration of copper nanoparticles, 150%, the highest inhibition zone was 33 mm against *Staph.saprophyticus*, while the lowest inhibitory activity was in the inhibition zone of 21 mm, also against *Burkhoderia cepacia.*

Table 3: The antimicrobial activity of CuO from extracellular sources on MRSA bacteria found in UTIs and their inhibitory zone

Concentration % / Bacteria species	75	100	150
	Diameter of inhibition zone (mm)		
Klebsiella pneumonia	25	25	26
Citrobacter.koseri	22	23	25
Streptococcus.agalactia	20	23	26
Staphylococcus.waxenri	25	24	27
Staphylococcus.hominis	30	30	31
Staphylococcus.saprophyticus	30	32	33
Burkhoderia.cepacia	20	19	21
Escherichia.coli	21	21	22
Enterobacter.aerogenes	23	22	21
Staphylococcus.aureus	25	25	26
Proteus.mirabilis	24	21	21

DISCUSSION

Antibiotic resistance in Enterobacteriaceae and certain gram-positive cocci demonstrates substantial changes in traits due to the widespread occurrence of RTF. β-Lactamase is expressed by the majority of resistance genes, yet some bacteria may exhibit resistance from other classes, as they pick up resistance genes from their surroundings or from other bacteria[18]. Some of β-lactamases have been imaged from the chromosomally encoded genes that occur spontaneously in some species to conjugative plasmids increasing their spreading ability among other species. RTF can be conveyed to drug-sensitive strains through conjugation, demonstrating kinetics similar to F transfer in *E. coli*[21].

The variation in inhibitory width may result from differing interactions between CuO nanoparticles and the microorganism, as well as the susceptibility of the bacteria employed in this investigation. The primary mechanism of toxicity of CuO nanoparticles, potentially linked to metal oxides, involves a positive charge that interacts with negatively charged microorganisms, resulting in electromagnetic interactions that induce oxidation and ultimately lead to microbial death. The bactericidal efficacy of CuO nanoparticles against bacteria is critically significant due to the potential of pathogenic bacteria to integrate into the ecosystem's food chain[22,23]. The antibacterial efficacy of CuO against bacteria has been established and reported in contemporary research. The nanoparticles may interact with the fundamental biological components, specifically sulfur and phosphorus[24,25]. As a result of nanoparticles, this mechanism has the potential to modify the phosphorus and sulfur content of genomic DNA. This would result in the inhibition of DNA replication, which would ultimately lead to the elimination of microbes[26]. The findings were consistent with the findings of other researchers who demonstrated that TiO2 nanoparticles had antibacterial activity when exposed to visible light [27]. Furthermore, the findings revealed that oxide nanoparticles were the nanoparticles that were the most biocompatible and antibacterial.

CONCLUSION

In this study, it was effectively established that copper oxide nanoparticles may be successfully generated from a simple and inexpensive nanocrystalline CuO NPs powder by using extracellular components produced from environmental isolate *Lactobacillus plantarum* as a reducing agent. This was accomplished through the development of a biosynthetic process. Following the completion of an antibacterial activity test, it was demonstrated that the bio-synthesized material exhibits a powerful antibacterial activity against the bacteria that were first introduced. It was discovered that the largest inhibition zone against *Staph. saprophyticus*, and the lowest inhibition zone against *Burkhoderia cepacia*.

REFERENCES

1. Zago M, Fornasari ME, Carminati D, et al. Characterization and probiotic potential of *Lactobacillus plantarum* strains isolated from cheeses. Food Microbiol 2011;28:1033.
2. Li S, Tang S, He Q, et al. Changes in proteolysis in fermented milk produced by *Streptococcus thermophilus* co-cultured with *Lactobacillus plantarum* or *Bifidobacterium animalis* subsp. *lactis* during refrigerated storage. Molecules 2019;24:3699.
3. Kanmani P, Kumar RS, Yuvaraj N, et al. Probiotics and their functionally valuable products: A review. Crit Rev Food Sci Nutr 2013;53:641.
4. Zhang WX. Nanoscale iron particles for environmental remediation: An overview. J Nanopart Res 2003;5:323.
5. Shaheen G, Akram M, Jabeen F, et al. Therapeutic potential of medicinal plants for the management of urinary tract infection: A systematic review. Clin Exp Pharmacol Physiol 2019;46(7):613-624.
6. Shah MA, Kassab YW, Farooq MJ, et al. Recent studies on urinary tract infections in diabetes mellitus. Health Sci J 2020;14(3):0.
7. Kalal BS, Nagaraj S. Urinary tract infections: A retrospective, descriptive study of causative organisms and antimicrobial pattern. Germs 2016;6(4):132.
8. Frost F, Craun GF, Calderon RL. Increasing hospitalization and death possibly due to *Clostridium difficile* diarrheal disease. Emerg Infect Dis 2010;8(8):619-625.
9. Lynch SV, Dixon L, Benoit MR, et al. Role of the rapA gene in controlling antibiotic resistance of *Escherichia coli* biofilms. Antimicrob Agents Chemother 2007;51:3650-3658.
10. Kolar M, Urbanek K, Latal T. Antibiotic selective pressure and development of bacterial resistance. Int J Antimicrob Agents 2001;17:357-363.
11. Leber AL. *Clinical Microbiology Procedures Handbook*. 4th ed. ASM Press; 2016.
12. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. 32nd ed. CLSI Supplement M100; 2024.
13. Saleh RF, Al-Sugmiany RZ, Al-Doori MM, Al-Azzawie A. Phenotypic and Genetic Effects of Wi-Fi Waves on Some Bacterial Species Isolated from Otitis Media Infection: doi.

Accepted

org/10.26538/tjnpr/v4i12. 6. *Tropical Journal of Natural Product Research (TJNPR)*, 2020; *4*(12), 1056-1063.

14. Yeong MS, Hee MS, Choon CH. Characterization of high-ornithine-producing *Weissella koreensis* DB1 isolated from kimchi and its application in rice bran fermentation as a starter culture. Foods 2020;9:1545.
15. Lee IC, Caggianiello G, van Swam II, et al. Strain-specific features of extracellular polysaccharides and their impact on *Lactobacillus plantarum*-host interactions. Appl Environ Microbiol 2016;82:3959.
16. Yaaqoob LA. Evaluation of the biological effect of synthesized iron oxide nanoparticles on *Enterococcus faecalis*. Iraqi J Agric Sci 2022;53:440.
17. Machado I, Graça J, Lopes H, et al. Antimicrobial pressure of ciprofloxacin and gentamicin on biofilm development by an endoscope-isolated *Pseudomonas aeruginosa*. ISRN Microbiol 2013;2013:178646.
18. Dhoondia ZH, Chakraborty H. *Lactobacillus*-mediated synthesis of silver oxide nanoparticles. Nanomater Nanotechnol 2012;2:15.
19. 19.Saleh, R. F., & Gaidan, A. M. (2021). Biosynthesis and characterization of silver nanoparticles using Cinnamomum zeylanicum extract and a study of antibacterial effect against multi-drug resistance Gram-negative bacteria. *Biomedicine*, *41*(2), 249-255.
20. Sultan, A. H., & Saleh, R. F. (2025). Bioactivity of Gold Nanoparticles Synthesized from Lion's Mushroom on Multidrug-Resistant (MDR) ESKAPE Bacterial Isolates. *Tikrit Journal of Pure Science*, *30*, 1.
21. Prodan AM, Iconaru SL, Chifiriuc CM, et al. Magnetic properties and biological activity evaluation of iron oxide nanoparticles. J Nanomater 2013;2013:893970.
22. Mahdavi M, Namvar F, Ahmad MB, et al. Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using *Sargassum muticum* aqueous extract. Molecules 2013;18:5954.
23. Qader, Q. A., Noumi, B. S., & Saleh, R. F. (2021). Antibacterial effect of biosynthesis silver nanoparticles on Pseudomonas aeruginosa. *Tikrit Journal of Pure Science*, *26*(5),22-26.
24. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir 2002;18:6679.
25. Wang J, Zhou G, Chen C, et al. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 2007;168:176-185.
26. Maaroof M, Mahmood A. Determination of the immunogenic and hematologic effects of titanium nanoparticles manufactured from *Aspergillus flavus* in vivo. Jordan J Biol Sci 2019;12(2):135-140.
27. Gelover S, Gómez LA, Reyes K, Leal MT. A practical demonstration of water disinfection using TiO_2 films and sunlight. Water Res 2006;40:3274-3280.

Accepted