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Antibacterial Activity of Curcumin-Loaded Copper Nanoparticles Against Multidrug-Resistant UTI Causing *Staphylococcus Aureus*

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Abstract: The production of biofilms by bacteria like *S.aureus* is frequent, and it leads to various treatment resistance. Plants and vegetables have more benefits in the green synthesis of nanoparticles (CuNPs) since they are safe to handle and easily accessible. The objective of the present study is to analyze the antibacterial activity of Curcumin-loaded Copper Nanoparticles [Cur-Cu-NPs] against *S.aureus*, which causes multidrug-resistant UTIs. Physical analysis (Scanning Electron Microscope [SEM]) and Chemical analysis (X-Ray Diffraction [XRD] and Fourier Transform Infrared [FTIR]) have all been used to validate the synthesis of Cur-Cu-NPs. The discovery of virulence factors that might be used as targets for vaccine and therapeutic development is a critical stage in this process. Curcumin, a ready-made compound which act as reducing and capping agents in the conversion of silver nitrate to CuNPs. The function of biomolecules in bioreduction and effective stabilization of CuNPs was established by FTIR. The crystalline character of the produced CuNPs with [FCC] Face centered cubic structure may be seen in XRD patterns. CuNPs produced with an average particle size of 140-190 nm had a spherical form, according to SEM examination. The efficacy of green-generated nanoparticles derived from aqueous extract against the UTI-causing *Staphylococcus aureus* strain was assessed in the clearance of micro titre wells during Minimum Inhibitory concentration analysis in µg/ml. These green synthesized Copper nanoparticles had significant antibacterial activity. The present study is supportive evidence that the synergistic additive medications result in best bactericidal actions with fewer adverse effects.

Keywords: Curcumin, Copper Nanoparticles, Phytocompound, Physical and Chemical analysis.

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1. INTRODUCTION

Nanotechnology allows researchers to examine the biological features of well-known recognized antibacterial agents by modifying their morphological features. Recent advancements in nanotechnology, notably the ability to make highly organized microparticles of any size and shape, have resulted in the development of new biocidal agents.[1] Researchers have sought to develop innovative, cost-effective, and resistant-free antibacterial reagents. Such concerns and requests have inspired a resurgence in the use of nanoparticle-based medications, which may have a broader spectrum of activity than antibiotics and are less likely to cause microbial resistance. Copper and other inorganic nanocrystalline metal oxides are especially intriguing because they are made with very high surface area and volume ratio, which suits for various pharmacological applications. Inorganic microbicidal materials outperform organic antibacterial chemicals in terms of durability, toxicity, selectivity, and heat resistance. [2,3] *Staphylococcus aureus* is a gram-positive coccus that causes a nosocomial infection. Infection with Staphylococci is almost often followed by a significant amount of pus accumulation. Boils, folliculitis, pneumonia, acute enteritis, burn infections with bacteremia, scaling skin syndrome, and toxic shock syndrome are all caused by staphylococci, which account for more than 80-90 percent of all suppurative infections. [3] Due to the development in the resistance rhythm of this bacteria to multiple antibiotics, the antibiotic era, which is just 60–80 years old, is also under threat.[4] MRSA infection has resulted in a rise in comorbidity and mortality in recent years, necessitating further research on how to manage these infections. A range of variables contributes to the drug resistance and severity of staphylococcal infections in hospitalized patients, with biofilm being the most virulent. [5] Biofilm formation is seen as a detrimental component since bacteria grow mostly from suspended populations. Antimicrobial drugs and the human immune system are blocked by biofilm, which prolongs the time that bacteria may colonise an area. [6] Throughout time, *S. aureus* has developed and gained a range of techniques to evade the human immune system and resist antibiotic therapy. As a result, MRSA has developed, and strains linked with health care (HA) and community (CA) have emerged (CA). MRSA has presented a danger to humanity's civilization. All-lactam drugs are resistant to MRSA, and some strains are resistant to several antibiotics. Resistance to all known antibiotic classes has evolved within the species because of mutation and horizontal gene transfer, raising concerns about the availability of effective chemotherapeutic treatments in the future. During the 1990s, the prevalence of MRSA increased dramatically in several countries.[7] Metallic nanoparticles, particularly copper nanoparticles (CuNPs) generated by bioengineered sources, have been intensively investigated in the search for alternative therapies for infections and other illnesses. The goal of this research is to see if Phytochemicals from *Curcuma longa* and CuNPs have a synergistic impact. Antibiofilm and antibacterial activities against multi-drug resistant uropathogenic *S.aureus* isolated from urinary tract infections were also used to test the nanoparticles' biological activity. UV-Visible, XRD, FTIR, and SEM techniques were used to analyze the phytochemical capped Copper nanoparticles [Cur-CuNPs].

2. MATERIALS AND METHODS

2.1. Isolation of Staphylococcus aureus

The Microbiology laboratory of Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, undertook this 7-month prospective investigation.

2.2. Sample size

This study would be performed for a period of six months. Hence, Continuous sampling from exudates has been done.

2.3. Culture

The enrichment media blood agar (BA) is used to isolate pathogenic gram-positive bacteria such as *Staphylococcus aureus*. Small colonies of pathogenic *Staphylococcus aureus* surround clean zones on BA. The reason for this zone is because *Staphylococcus aureus* haemolyzes the blood in the medium, which is referred to as beta hemolysis. A disc diffusion assay was used to establish the presence of methicillin-resistant S. aureus (MRSA). Mueller-Hinton agar medium with 2% NaCl and Cefoxitin antibiotic discs (6 µg/mL) was used and kept for incubation at 37 °C for 24 hours. Clinical Laboratory Standards Institute (CLSI) criteria were used to interpret the growth inhibition zones. [8]

2.4. Materials

Sisco Research Laboratory, Chennai provided copper nitrate (CuNO3), gallic acid, curcumin (purity > 80%), polyvinylpyrrolidone (PVP), and pluronic F-127.

2.5. Preparation of Curcumin – Copper nanoparticles

Cur-CuNPs were made utilizing an antisolvent crystallization approach, which is the synthesis method for a medication that is weakly water-soluble. Curcumin is dissolved in an organic solvent before being added to deionized water while being constantly stirred in this technique of production.

2.6. Characterization

The presence of reflection was verified by XRD patterns and a detailed examination of the form, size, and distribution of the synthesized Copper nanoparticles from curcumin was documented by the Scanning Electron Microscopy (SEM) equipment. In the synthesis and stabilisation of nanoparticles, FTIR analysis was employed to discover possible functional groups.

2.7. Biofilm assay

2.7.1. Tissue culture plate method

In 10 mL of trypticase soy broth, the organisms were inoculated and kept for incubation at 37^0C for 24 hours after being isolated from new agar plates. The cultures were then diluted in new media at a ratio of 1:100. 200 µl of diluted cultures were placed in individual wells of sterile microtitre plates. Positive and negative control wells were preserved for incubation and contained inoculated sterile broth. Biofilm generated by bacteria clinging to the walls was dyed with crystal violet and fixed with 2% sodium acetate (0.1 %). Using ELISA auto reader Evolis twin plus at 560 nm, the optical density (OD) of stained adherent biofilm was determined. [9]

2.7.2. *Minimal inhibitory concentration (MIC) determination*

Cur-Cu-NPs antibacterial activity was tested using the conventional broth dilution technique. Accordingly, the MIC was calculated using a micro broth dilution technique in Nutrient broth (Hi-Media) utilizing successive two-fold dilutions of Cur-Cu-NPs in concentrations ranging from 2 - 2500 g/ml, initial bacterial inoculums, and a period and temperature of incubation of 24 hours at 37 °C.[10]

3. RESULTS

MRSA was found in 60 of the 250 skin and soft tissue infection samples.

3.1. Biofilm test

Compared to the tube method and Congo red agar, the tissue culture plate is a more precise and reliable method for the detection of staphylococci that produce biofilms. Consequently, it is still applicable as a screening technique for biofilm detection. By evaluating the optical densities of dyed bacterial films adhered to the bottoms of plastic tissue culture plates, staphylococci adhesion to smooth surfaces was evaluated. The weight of the adhering bacterial film was linked with the optical densities ($r = 0.906$; $P<0.001$). Additionally, the data matched ocular evaluations of bacterial adhesion to culture tubes, microtiter plates, and tissue culture plates. For an antibacterial assay, specific clinical strains were put through the broth dilution procedure. Figure 1 depicts the effects of MRSA bacteria on biofilm development.

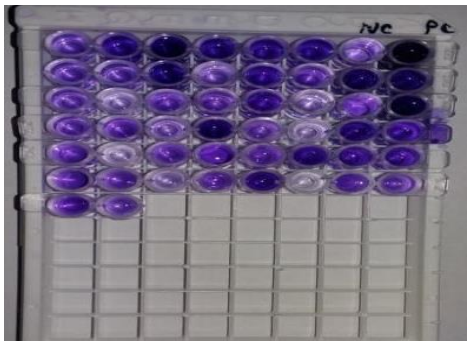

Fig 1: Biofilm formation using tissue culture plate method

We examined how biofilm development, a primary virulence factor in bacteria, affected the results. Table 1 summarises the findings. Sixty *Staphylococcus aureus* strains were found to generate biofilms and be methicillin-resistant out of 250 obtained from clinical samples. Methicillin-resistant bacteria are linked to higher rates of morbidity and mortality. Infections caused by *Staphylococcus aureus* (MRSA) are on the rise, prompting further study into infection management. Eighteen have been identified as potent biofilm producers.

Table: 1 Biofilm producers of 50 MRSA

No. of. MRSA	Strong	Moderate	Weak
60	18	26	16

3.2. Characterization studies

3271, 2922, 1634, 1532, 1404, 1235, 1024, 874, 533, 400 cm^{-1} were the peaks recorded from Cur-Cu nanoparticles. According to the findings, 3271 cm^{-1} was associated with OH stretching vibration of alcohol, 2922 cm-1 with CH stretching vibration of an aromatic compound, 1634 cm^{-1} with C=O stretching of carboxylic acid, biomolecules such as carbohydrate and protein, 1404 cm^{-1} with CC bond in the aromatic ring, 1532 NH of the amide linkage, 1235 with CN stretching of amines, 1024 with COC, 874 with CH, 533 with CH, 1024 with COOH. [Figure 2].

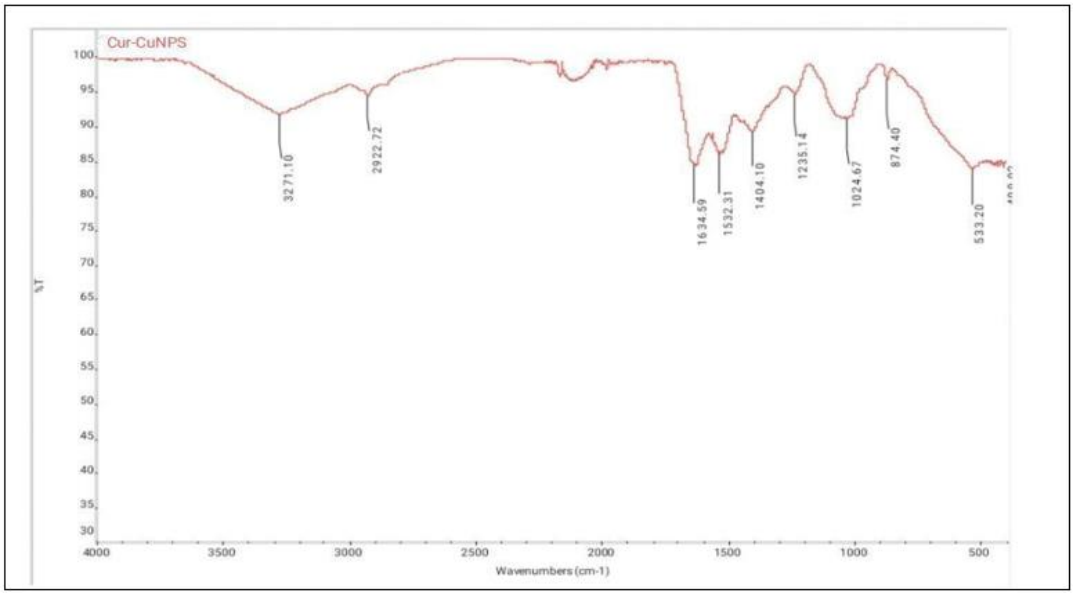

Fig: 2 FTIR Spectra of Green Synthesized Nanoparticles

The XRD patterns of dried $CuNO_3$ produced Copper nanoparticles interacting with curcumin reveal a distinctive peak (at 2θ = 38.325°, 46.43°, 57.73°, and 85.98°) with a number of Bragg reflections [Figure:3].

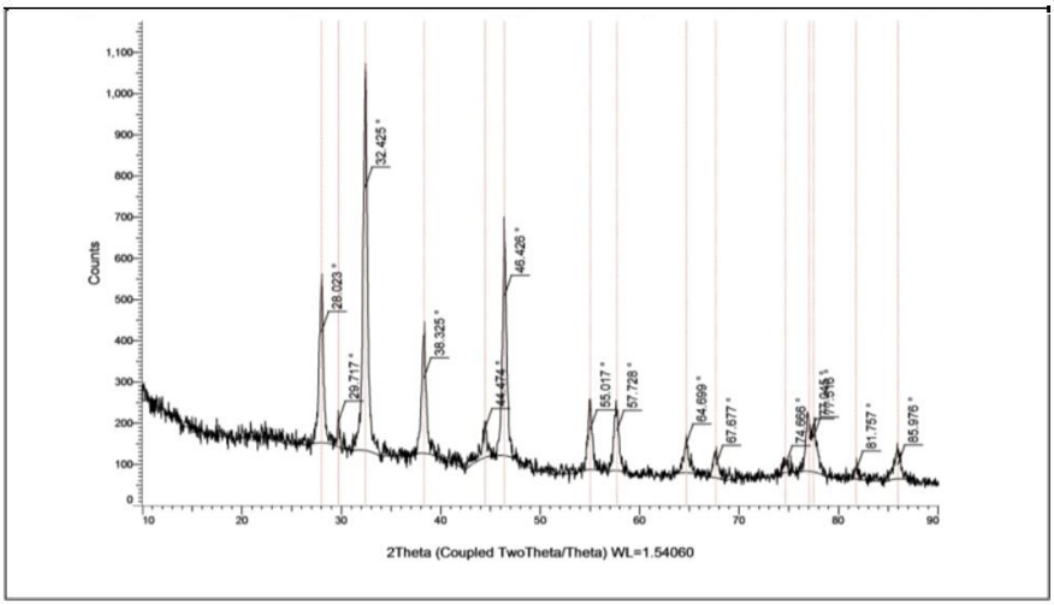

Fig: 3 XRD Spectra of Green Synthesized Nanoparticles

SEM scans revealed the morphological character and size details of the green produced copper nanoparticles coupled with curcumin. From the SEM picture, the size of the nanoparticles was determined to be between 140 and 190 nm. [Figure: 4]

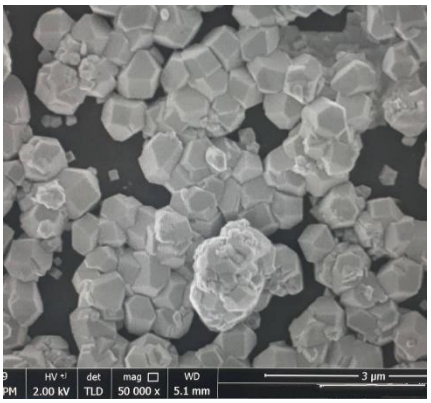

Fig :4 SEM images of Green Synthesized Nanoparticles

3.3. *MIC determination*

In vitro antibacterial activity of Cur-Cu-NPs was tested on 12 strong MRSA biofilm producers. Figure:5 illustrates that by using the micro broth dilution procedure, the MIC was identified. It was also investigated how Cur-CuNps affected the kinetics of these bacteria's growth. Bacterial culture media that had been inoculated with Cur-CuNps were checked to see if any bacterial growth was seen after being incubated for an overnight period.

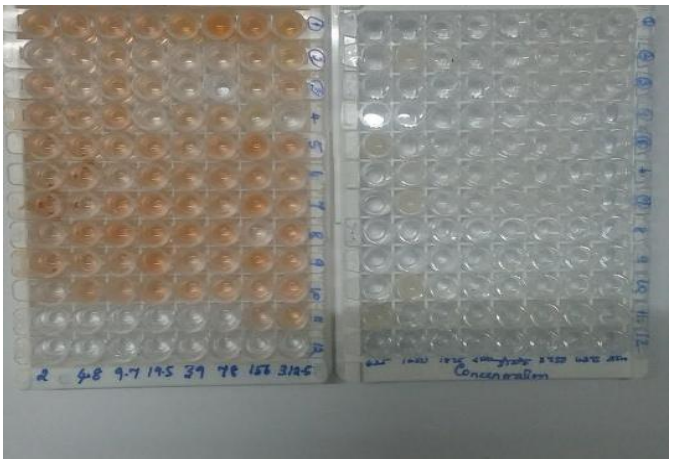

Fig: 5 MIC result

Bacteria were significantly destroyed by Cur-Cu-NPs. MRSA strains were destroyed 71% ($P \leq 0.001$) after 6 hours of incubation at 312.5, 625, and 1250 µg/ml concentrations, respectively, (Table: 2) whereas roughly 92 % of MRSA were eliminated after 24 hours of incubation. Figure:5 shows the MIC determination of MRSA that produces robust biofilms, as well as positive and negative controls. There was a considerable negative association ($r2 = 0.75$) between the inhibition zone discovered in disc diffusion tests and the MIC/MBC determined from liquid cultures with various strains in the case of curcumin-capped copper nanoparticles. For S. aureus, there was no strain-specific variation in MIC/MBC, whereas, for E. coli, there was significant strain-specific variation.

Table: 2 Antibacterial isolates of Cur-Cu-NPs against clinical isolates of MRSA

Biofilm producing MRSA (18)	MIC (µ g/ml)	Biofilm producing MRSA (18)	MBC (µ g/ml)
4	312.5	3	312.5
8	625	8	625
6	1250	7	1250

4. DISCUSSION

The zoonotic pathogen Methicillin-resistant Staphylococcus aureus (MRSA), which is significant for public health and veterinary purposes, is a serious human pathogen. Food poisoning, pyogenic endocarditis, suppurative pneumonia, otitis media, osteomyelitis, and pyogenic infections of the skin and soft tissues are among the serious infectious illnesses that MRSA frequently causes in people. Misuse and overuse of antibiotics can be prevented by constant monitoring of the antibiotic susceptibility testing for the bacterial isolates in the hospital and by framing antibiotic policy and initiating antibiotic stewardship program. Due to its many advantages, this organic science method to the mixture of copper nanoparticles is a very important effort being cared to in nanomedicine. Due to its high practicality and eco-friendliness, curcumin may be used in nanotechnology preparation projects to combine copper nanoparticles in a wide range of ways. MRSA was found in 60 of the 250 skin and soft tissue infection samples. In the study by N. Sobhy et al.,[11] A total of 60 isolates from diverse skin disorders were collected. *Staphylococcus spp*. accounted for 42%, *Streptococci spp*. 4%, and *Proteus spp*. 4% of all infections. In 38 of the 42 staphylococci, *S. aureus* was discovered (90.48%). Eighteen have been identified as potent biofilm producers in the present study. 43 clinical isolates of the S. Using common microbiological methods, 150 pus samples contained aureus. In 30 (69.8%) and 28 (65.1%) of the S. isolates, biofilm development was seen. aureus using the TM and TCP methods, respectively. S. that creates biofilm When compared to the biofilm nonproducers, aureus showed a greater rate of antimicrobial resistance ($P<0.05$). An important finding is that 86.7% of S. The biofilm nonproducers all lacked multidrug resistance (MDR), whereas aureus did ($P<0.05$). MRSA was discovered in large percentages (43.3%) of biofilm producers, but not in any of the biofilm nonproducers ($P<0.05$). [Neopane P et al., 2018] [12] The FTIR data showed that bioactive functional groups were acting as capping and reducing agents. The crystalline character of the CuNPs was verified by XRD spectra. According to Kamble et al., 2016, the high peak at 3508 cm^{-1} indicates the presence of a hydroxyl group, while the closely spaced doublet at 1628 and 1600 cm^{-1} is due to symmetric and unsymmetrical stretching of the alkene conjugated to the 1,3-diketone group. The peak at 1501 cm^{-1} is due to the stretching vibration of the aromatic ring. In the FTIR spectra of Cu-NPs, the peak at 3501 cm^{-1} with the increased absorption intensity of the hydroxyl group is clearly visible.[13] The vibrational stretches at 1651 and 1310–1015 cm −1 are seen in green synthesized copper nanoparticles in the study of Shankar et al., 2014 [14] The standard XRD pattern of curcumin-assisted Cu NPs (CuC) is discovered in a study by Chandran et al., 2022, which reveals the formation of pure metallic Cu with crystallinity, with a prominent peak at $2\theta = 43°$ oriented along the (111) plane. The other peaks indexed at (200) and (220) planes are $2\theta = 50.32°$ and 74.10°, respectively.[15] The coordination of CU enolic-OH with Cu-NPs might explain the higher frequency. Scanning electron

microscopy (SEM) study of CU-NPs also helps with nanoparticle synthesis. Nanoparticles have a small rod-like structure with a significant number of patches on the surface, according to MA Asghar et al., 2020 [16] The interaction between gram-positive and gram-negative bacteria and copper nanoparticles was previously reported to be stronger for gram-positive bacteria than for gram-negative bacteria. [17] *P. aeruginosa* and its cell wall, which is made up of lipids, proteins, and lipopolysaccharides (LPS), offers effective defense against biocides. However, gram-positive bacteria like *S. aureus* do not have LPS in their cell walls. The different varieties of bacteria's resistance to nanoparticles will vary depending on their physiology, metabolism, and level of interaction with them. Copper oxide nanoparticles' MIC and MBC values against MRSA were discovered at concentrations of 800 and 1600 g/ml, respectively. [18] Another study by S. Malathi et al., 2022 used an environmentally friendly and low-cost technique to investigate the antibacterial efficacy of a mixture of AgNPs and curcumin nanoparticles (Cur-NPs) against Gram-negative e.coli at 100 g/mL. Furthermore, when doses of 40 g/mL curcumin and 5 g/mL Ag were used, this combination therapy (Cur-SNPs) shown greater ability to totally inhibit the growth of e.coli biofilm. Surprisingly, no inhibitory movement on biofilm was observed using either Cur-NPs or AgNPs alone at this point.[19]

5. CONCLUSION

The current research shows that MRSA is a robust biofilm developer and that treating it with antibiotics caused difficulties. As a result, newer generating medications such as Cu nanoparticles can be utilized to successfully control germs and prevent MRSA infections. Curcumin mediates the reduction of CuNO3 into CuNPs. Bacteriostatic characteristics are seen in CuNPs. CuNPs-curcumin exhibit synergy and addictive behavior, this conjugation is usually favorable since bacteria cannot develop resistance to this consortium.

6. ACKNOWLEDGMENT

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7. ETHICAL APPROVAL STATEMENT

The study was approved by the Institutional Ethics Committee – SBCP/IRB/098

8. AUTHORS CONTRIBUTION STATEMENT

Kamala contributed significant contributions to the conceptualization, data collection, and drafting of the work, as well as key revisions for important intellectual content. Ishwarya and Manipriya completed Characterization investigations and final acceptance of the published version, and agreed to be responsible for all parts of the project.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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