Hindawi
Journal of Nanomaterials
Volume 2023, Article ID 5668025, 7 pages
https://doi.org/10.1155/2023/5668025

Research Article

Synthesis and Antimicrobial Activity Assay of Nanometal Oxide-Doped Liquid Crystal

S. Deepthi[1], **S. Srikiran**[2], **M. Satyanarayana Gupta**[3] **and Adamu Mulatu Kumara**[4]

[1]*Department of Science and Humanities, Lendi Institute of Engineering and Technology, Vizianagaram, India*
[2]*Centre for Nanotechnology, Andhra University College of Engineering, Visakhapatnam, India*
[3]*Department of Aeronautical Engineering, MLR Institute of Technology, Hyderabad, Telangana, India*
[4]*College of Engineering and Technology, Wollega University, Nekemte, Ethiopia*

Correspondence should be addressed to Adamu Mulatu Kumara; adamum@wollegauniversity.edu.et

Received 30 August 2022; Revised 21 October 2022; Accepted 27 January 2023; Published 7 April 2023

Academic Editor: B. Deepanraj

The spread of infectious diseases across the globe as a result of numerous bacterial and fungal variations has become a serious threat to human life. A critical global situation is the need to search for antibiotic resistance and develop new treatments. The most crucial role is for academics to advise the pharmaceutical industry about substances with antimicrobial assessments. One of the challenges in implementing novel medicine delivery is the discovery of compounds having antibacterial and antifungal characteristics against Gram-positive and Gram-negative bacteria and fungi. The superiority of the metal oxide-doped liquid crystal technology for antibiotic resistance is revealed. In this work, a bacterially triggered drug delivery system using a nanometal oxide-doped lipid-based liquid crystal system was explored. Copper oxide (CU) and cholesteryl stearate (CS) are processed using ultrasonication to produce the complex chemical in powder form (CSCU). Functional groups are predicted by Fourier transform infrared (FTIR) analyses, while surface appearance and dimensions that support the compound CSCU's biological characteristics are revealed by scanning electron microscope (SEM) and transmission electron microscope (TEM) analyses. Using the agar-well diffusion method, this substance was tested for antibacterial and antifungal activities against the Gram-positive bacteria *Streptococcus pyogenes* at various concentrations ranging from 0.6 to 1.25 μg/ml. Additionally, this substance exhibits a range of moderate to good antifungal efficacy against *Aspergillus niger*.

1. Introduction

Infections with bacteria and fungi can have a mild to serious impact on human health. These microorganisms exist on the mucosal epithelial surfaces of human bodies, causing infections [1]. Many diseases, from minor infections to infectious, life-threatening infections, are caused by microbes. These microorganisms produce toxic shock syndrome and invasive infections that have significant morbidity and mortality rates. *Streptococcus pyogenes*, an exclusive human pathogen, is a genus of Gram-positive bacteria that causes a variety of illnesses that are extremely contagious. Hand-to-hand contact, nasal discharge, and even airborne droplets can all be ways that infections spread. The virulence factors produced by *S. pyogenes* as it spreads within the human body have an impact on the host immune system [2, 3]. The cause of several infections is also by the fungus *Aspergillus fumigatus*. It can be found everywhere in the environment, including in the dust, soil, and plant debris. This fungus produces conidia-like spores that are airborne. It is also one of the frequent causes of otomycosis, a condition that results in temporary hearing loss and ear pain [4, 5]. These bacteria and fungi spread into the human host system by creating colonies that have an impact on human health.

Rapid sequential scientific procedures are being used to conduct an in-depth investigation of the bacterial and fungal communities in a systematic manner. Due to the fact that bacteria and fungi can cause mild to severe diseases, the development of antimicrobials is necessary [6, 7]. The development of new medications to combat germs and fungus is time- and money-consuming. Drugs that are currently in the market could be expedited and made more affordable

through reformulation [8]. Both bacteria and fungi undergo repeated iterations of evolution over time. Therefore, it is vital to introduce new medications or reformulate existing ones with the appropriate admitters. Drugs that are efficient and cutting edge are being created in this procedure to combat bacterial and fungal infections and slow down or stop their growth [9]. Recently, nontoxic bioactive molecules attached to specific targeting ligands have been used to carry out the majority of work in the biological area.

The dual functions of lipid-based liquid crystals as carriers and drug delivery methods make them potentially useful in the pharmaceutical industry [10, 11]. Due to their hydrophobic and hydrophilic characteristics and their role as carriers for antimicrobial peptides, lipid-based liquid crystals play a significant role in biological phenomena [12]. These have a wide range of medical uses, such as drug delivery, carrier capsules, biocompatibility, and biodegradability [13, 14]. Because of its hydrophobic properties, which result in an antibacterial phenomenon, lipid-based liquid crystals have cellular storage and have become important in biological investigations. Cholesteryl stearate (CS) is an ester of cholesterol and stearic acid, which is a class of lipid-based liquid crystal [15, 16]. These bioactive compounds' excellent capabilities are released through nanocontainment [17]. Due to their outstanding achievements against bacteria and fungi, metal oxide nanomaterials were first appeared in the biological field. The effectiveness of metallic nanocrystals as antibacterial agents has been demonstrated in numerous researches [18, 19]. Copper oxide nanoparticles stand out among the metal oxide nanoparticles for their effectiveness in repelling microorganisms [20, 21]. This research focuses on the antibacterial properties of nanometal oxide-doped lipid-based liquid crystals and their active engagement as antimicrobial peptide carriers. Doping of nanoparticles into lipid-based liquid crystal compound results in the enhancement of physical, chemical, and biological properties.

In this study, ultrasonication method is used to mix the lipid-based liquid crystalline compound CS with the metal oxide nanocompound copper oxide in a specific mass to volume ratio. The combined molecular structure of CSCU is shown in Figure 1.

The compound's capacity for antibacterial testing is encapsulated by its supramolecular structure and hydrophobic character [22–24].

The complex CSCU has distinct spectral peaks that can be seen using Fourier transform infrared (FTIR) spectroscopy [25, 26]. High surface areas and crystal morphologies that have been verified by scanning electron microscope (SEM) and transmission electron microscope (TEM) studies are used in the preparation of CSCU [27–29]. The agar-well diffusion method was used to assess the potential antibacterial uses of the CSCU compound [30]. *S. pyogenes*, a Gram-positive bacteria, and *A. fumigatus*, a fungus, are both susceptible to the antibacterial and antifungal properties of this nanoliquid crystalline substance [31, 32]. Lipid-based liquid crystals have gained importance in the field of biology due to their compatibility. They are good in dissolution and are very feasible in biodrugs. Nano-doped lipid-based liquid crystals enhance the biological properties which could be observed through TEM and SEM studies. The compound used by us "CS" is a lipid-based liquid crystal which has withstanding biological properties and the nanocompound copper oxide also competes with the chosen liquid crystal in biological properties. Hence, the chosen compound (CSCU) promotes good antibacterial and antifungal properties, which is a proof for a new compound to be used.

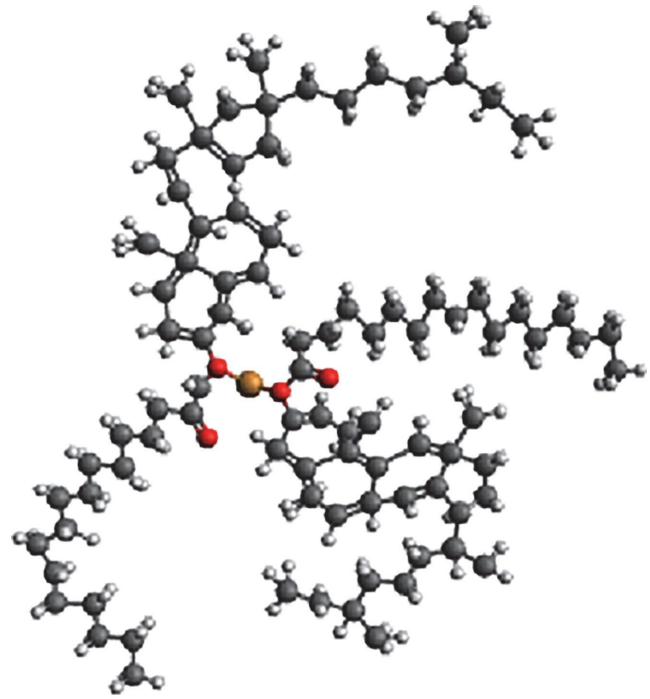


Figure 1: Molecular structure of CSCU.

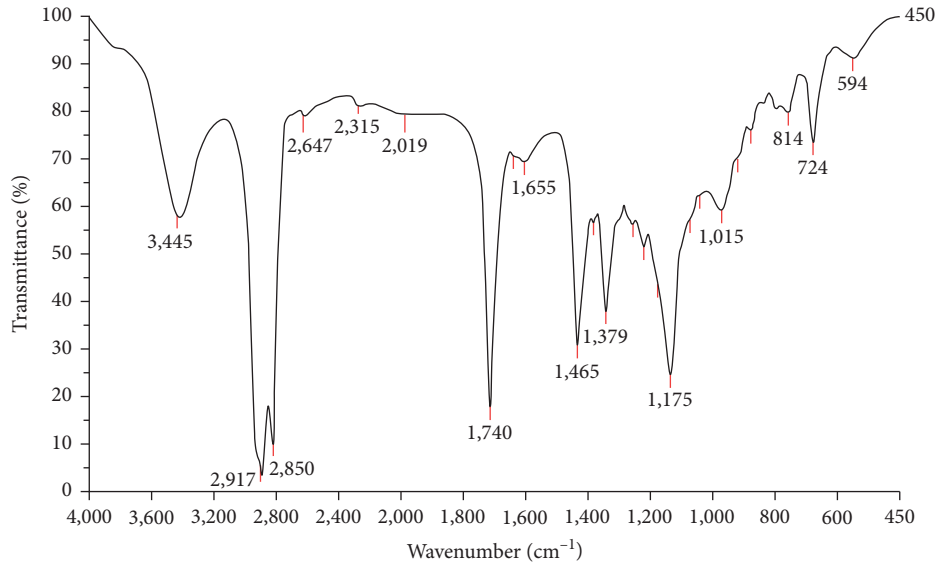
The compound CSCU with its antimicrobial activities shows prominence in drug delivery mechanism. Crystalline nature and equal surface morphological distribution of molecules in complex compound may have controlled release of drug.

2. Materials and Methods

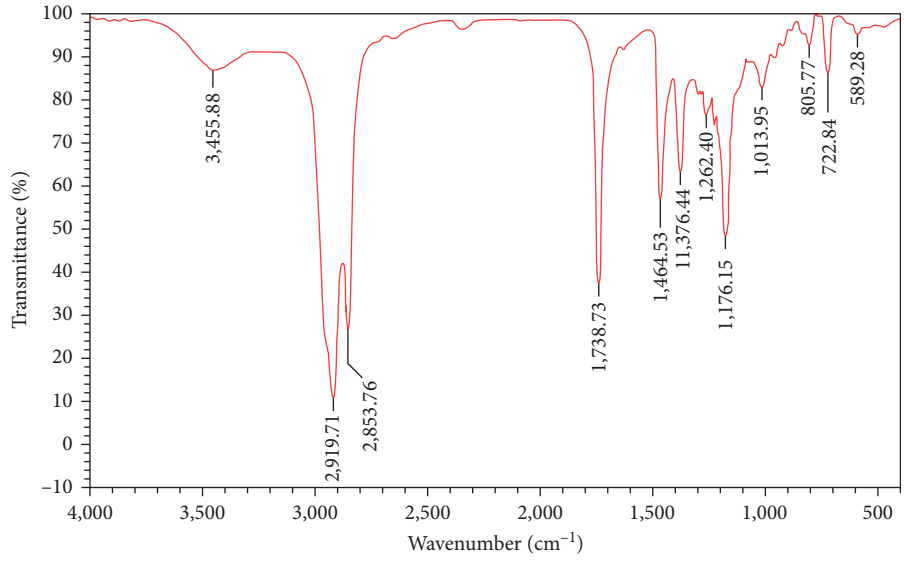
The various materials used for the purpose of experimentation and the methods for preparing the cultures are discussed in the continuing section.

2.1. Synthesis. Without additional purification, CS and copper oxide nanoparticles were employed after being obtained from Sigma-Aldrich. CS (500 mg) was dissolved in 15 ml of ethanol and sonicated for 10 min. In a separate beaker, copper oxide (0.012 mg) was dissolved in 5 ml of ethanol. The copper oxide solution was added dropwise to the ethanolic solution of CS and further sonicated for 30 min. Both substances were dissolved in an ethanol solution and ultrasonically processed using PCI Analytics. The reaction mixture was kept overnight and vacuum filtered and dried to obtain a solid precipitate [33].

2.2. Preparation of Bacterial Cultures. To test the antimicrobial activities of obtained powder (CSCU), agar-well diffusion method is used. In this method, 24 hr young cultures were



(a)



(b)

Figure 2: FTIR spectrum of (a) CS and (b) CSCU.

used to test the complex compound's antimicrobial activity. Microbes were cultivated after the media had been autoclaved for about 30 min at 1,200°C (15 psi). Nutrient agar medium, seeded with the appropriate strains of bacteria, was placed aseptically into each sterile petri dish, amounting to about 20 ml per plate. For solidification, the plates were kept at room temperature. A number 3 cup borer (6 mm in diameter) was used to create five uniform cups or wells in each petri dish once the material had solidified. Each cap's base had a drop of molten nutritional agar that had applied to seal it.

Young cultures with CSCU compound were used for 24 hr to test the antifungal activity using the agar-well diffusion method. An autoclave was used to sterilize the potato dextrose agar (PDA) medium for around 30 min. PDA medium in the amount of 20 ml (per petri dish), seeded with the appropriate fungal strains, was transferred aseptically into the petri dish. The plates were remained so that they could solidify.

3. Results and Discussion

The discussion below deals with the results associated with the work.

3.1. Spectral Analysis. The complex compound's FTIR spectra's spectrum analysis demonstrates that the crystalline lipid material and nanoparticles have been properly amalgamated. FTIR was used to conduct spectrum analyses on the complex chemical CSCU (Thermo Nicolet 6700). The bonding nature of CS and copper oxide has been confirmed through FTIR analyses

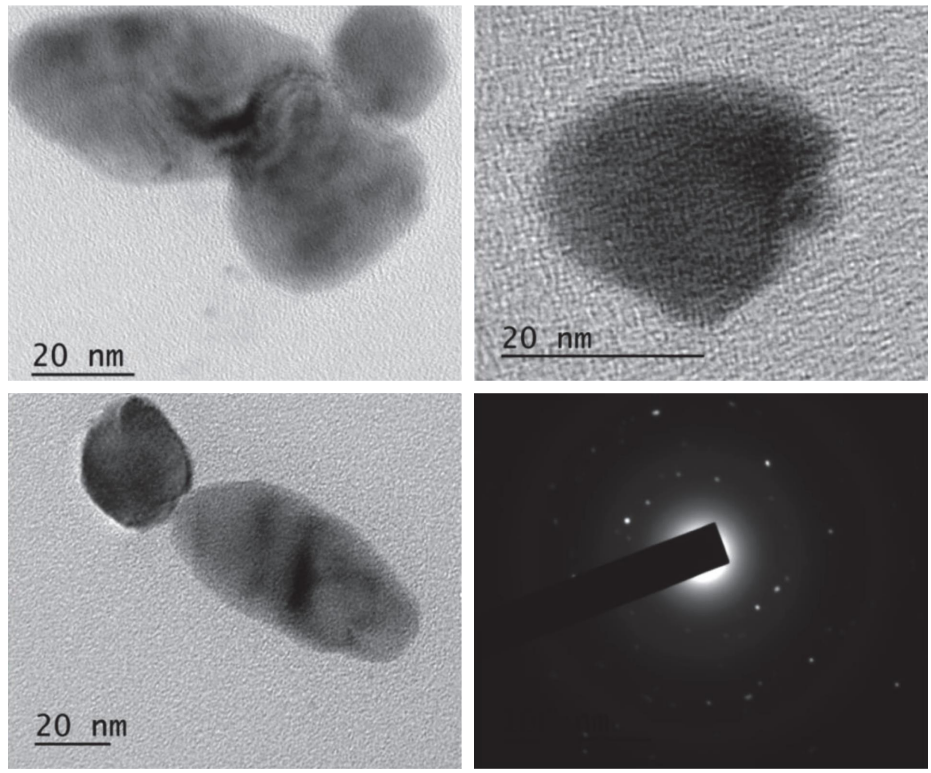


Figure 3: TEM analysis of CSCU.

of the complex compound (CSCU) from the shifts in both fingerprint and functional regions. The spectral wavenumbers between 400 and 4,000 nm were taken into consideration. The peaks that correspond to CS are 2,917, 1,415, and 840 cm^{-1}. By observing downward movements in C–H stretching and bending, copper oxide bonding was found to enhance the peaks. Also, C–O stretch was noticeable with a downward tilt. As shown in Figure 2, there is no discernible change in bonding when C=C and CH_3 are bent. Pyridine group, ketone group, and benzene ring structures make up the majority of original CS. Individual carbon atoms had an impact on the hydrophobicity of the CS during complex formation with metal oxide nanoparticles, which demonstrate an improved chemical potential in the alkyl group. From the peaks of FTIR, the amalgamation of CSCU compound is estimated and the same was interpreted through the structural evaluation.

3.2. TEM Analysis. Figure 3 shows TEM pictures of complicated compounds. TEM examination revealed the complicated compound's crystalline form. The concentration of both the compounds, CS and CU, is visible in TEM pictures, which also demonstrate an equal distribution of particles. The TEM image clearly shows that the complex molecule's morphology is practically spherical in shape. Figure 3 also shows that selected area electron diffraction (SAED) pattern reveals the complicated material's crystalline structure. Particles of synthetic CSCU are about 20 nm in size. The spreading of the substance into the petri dish with the nutrient agar media seeded is relatively simple, thanks to the special characteristics of nanocrystalline CSCU. Crystalline nature of the compound was revealed by TEM analysis. The significance of the biogenic material in terms of antimicrobial activities of complex compound strongly depends on its crystalline nature.

3.3. SEM Analysis. The SEM analysis of CSCU was performed on JSM-6390 for 5, 10, and 50 μm. Morphological studies were performed on complex compound to visualize the distribution of copper oxide nanocrystals on CS compound. The composition of elements can be known with topographical properties. The resolution of SEM images extends from 5 to 50 μm with magnification of 500–3,000x. As the magnification of the image increases, it reveals that CS and CU are well dispersed to form complex clusters, as shown in Figure 4.

SEM images demonstrate the appropriate cluster formation of the CSCU complex and its effectiveness as an antibacterial agent. Because the complex CSCU particles were equally dispersed, the substance had an antimicrobial effect on the *S. pyogenes* organism. The complex substance successfully eliminates the germs at higher concentrations and works down to lower ones. The complex substance demonstrates its perfection when antifungal activity against the *Aspergillus niger* bacterium is tested at different concentrations. Equally dispersed copper oxide nanoparticles on CS compound reveal the complex cluster of CSCU. As there is equal distribution of particles, the complex compound CSCU is effective on bacteria and fungi. The intricate cluster permits antifungal activity that is on par with industry norms.

3.4. Antibacterial and Antifungal Activities of CSCU. The complex compound CSCU was reconstituted with suitable solvent like dimethyl sulfoxide (DMSO) and tested at various concentrations (1, 2, 3, 4, 5, 6, 7, and 8 μg/ml). The test was

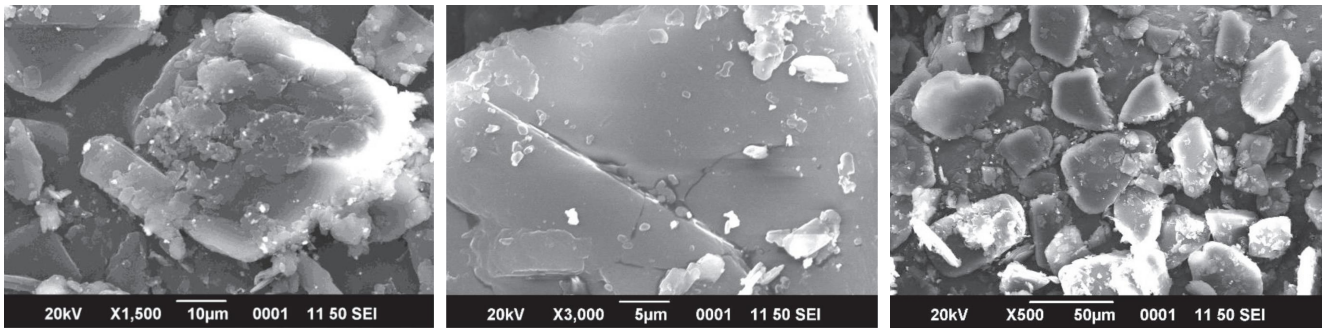


Figure 4: SEM analysis of CSCU.

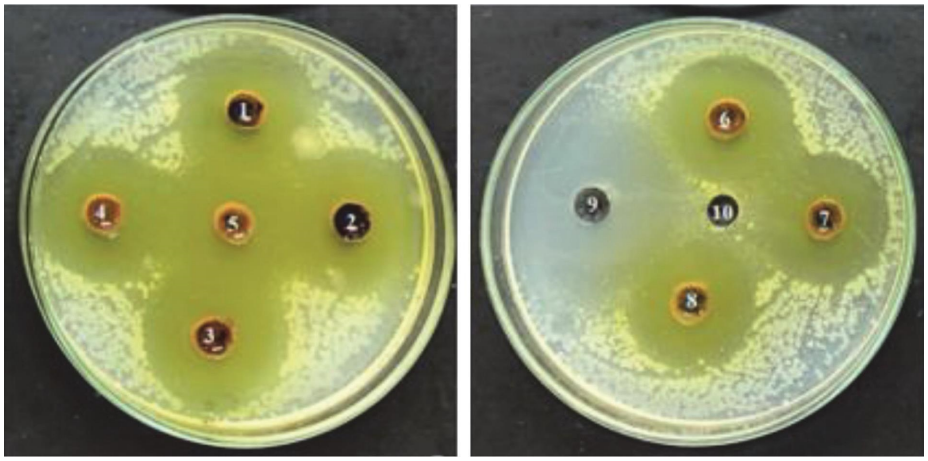


Figure 5: Antibacterial activity of CSCU.

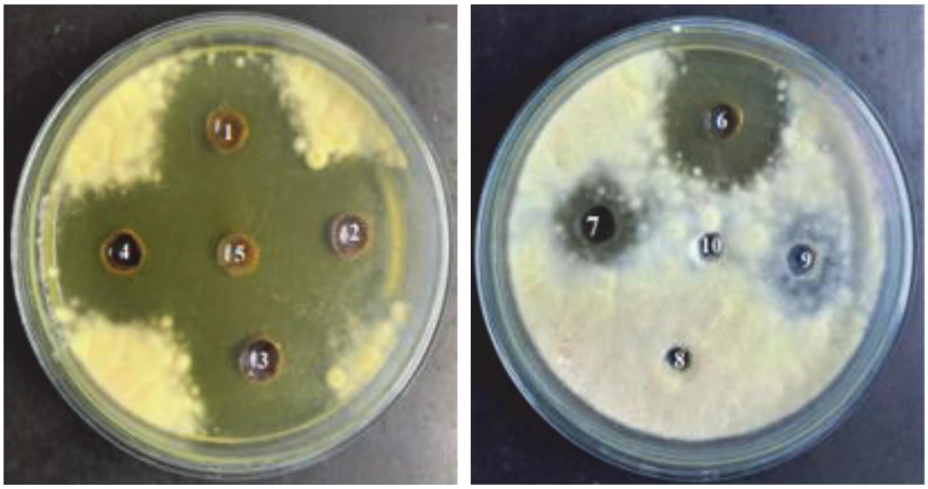


Figure 6: Antifungal activity of CSCU.

performed under control DMSO and standard antibiotic ciprofloxacin was carefully placed in the well. Petri plates were incubated at $37 \pm 2°C$ for about 12 hr. The $5\,\mu g/ml$ concentration was used as positive control. Figures 5 and 6 show the antibacterial and antifungal activities of the complex compound CSCU. The absence of bacterial growth is indicated by clear wells, as shown in Figure 5.

The chemical was reconstituted with DMSO, a suitable solvent, and added to petri dishes containing various bacterial and fungal strains at various concentrations.

3.5. Statistical Analysis. The zone of inhibition of *S. pyogenes* bacterial decay in petri dish of reconstituted compound (CSCU) at various concentrations along with the standard

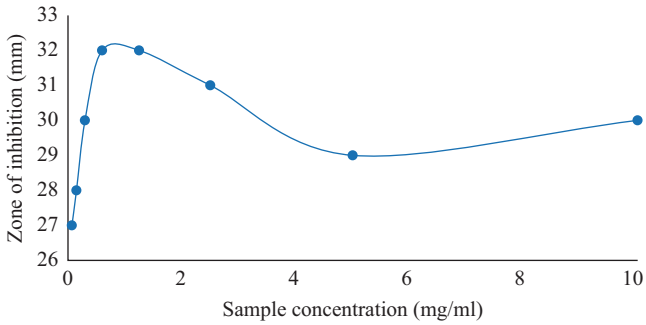


Figure 7: Concentration of sample against zone of inhibition.

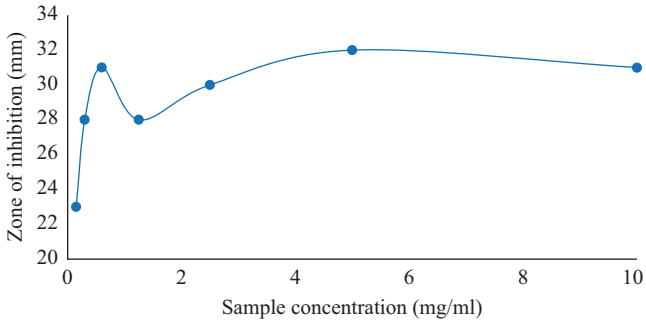


Figure 8: Concentration of CSCU against zone of inhibition.

ciprofloxacin and control DMSO in the incubated petri plates was incubated at $37 \pm 2°C$, as shown in Figure 7. The zone diameter in petri dish shows the active involvement of complex compound CSCU with concentration ranging from 0.6 to 1.25 μg/ml against bacterial strains.

The compound CSCU also exhibits antifungal properties. Strains of fungi at different concentrations are compared with standard values. The zone of inhibition of decay in petri dish of reconstituted compound (CSCU) at various concentrations along with the standard fluconazole and control DMSO in the incubated petri plates was incubated at $28 \pm 2°C$ about 24 hr. A standard with 5 μg/ml concentration was used as a positive control. The zone diameter in petri dish shows the active involvement of complex compound CSCU with concentration ranging from 2.5 to 5 μg/ml against fungal strains, as shown in Figure 8.

4. Conclusion

Due to the daily emergence of novel bacterial and fungal illnesses, drug delivery systems are concentrating on the development of various new substances. Utilizing an ultrasonication method, a liquid crystalline substance doped with nanometal oxide was created. Bonding between CS and CU is confirmed by significant wavenumber shifts with abrupt peaks in selected areas. By using the agar-well diffusion method, the hydrophobicity and supramolecular structure of CSCU well establish antibacterial and antifungal properties.

Lipid-based liquid crystals have gained importance in biology due to their compatibility. They have excellence in dissolution and are most feasible in bioavailability of drugs. Nano-doped lipid-based liquid crystals enhance their biological properties and could be observed through TEM and SEM studies. Our compound CS is a lipid-based liquid crystal which has withstanding biological properties and our nano-compound copper oxide also competes with the chosen liquid crystal in biological properties. So, our chosen compounds are properly sonicated to obtain the new compound (CSCU) which promotes good antibacterial and antifungal properties. Due to the individual performances of both on bacteria and fungi, we thought to have more pronouncement of the complex compound on these two that had obtained good results. The compound CSCU can further be tested for antioxidant and anticancer properties.

The development of a novel medication from the complex chemical CSCU is the main focus of this research.

Data Availability

The datasets generated during and/or analyzed during this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors thank the support extended by Centre for Nanotechnology, Andhra University College of Engineering, Visakhapatnam for allowing us to use their facilities and for recording infrared spectra, STIC, SEM, and TEM images. The authors also thank to Adhya Biotech Laboratories, Visakhapatnam for duly performing with hands on experience with agar-well diffusion method.

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