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Biosynthesis of Copper Oxide Nano Particles from Hibiscus Rosa Sinensis and its Efficacy Against Pseudomonas Aeruginosa

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Introduction: There is a growing interest in the production of metal nanoparticles utilizing eco-friendly techniques. This work investigates the potential of Hibiscus rosa-sinensis leaves for the green synthesis of copper oxide nanoparticles (CuO NPs). CuO NPs have exhibited substantial utility owing to their distinctive characteristics. The objective of this study is to investigate the efficacy of Hibiscus rosa-sinensis in synthesizing CuO NPs and to evaluate their ability to suppress biofilm formation by Pseudomonas aeruginosa. Methods: CuO NPs were synthesized by a biological process involving the leaves of Hibiscus rosa-sinensis. The presence of CuO NPs was confirmed by the observed color change from green to brown, which can be attributed to surface plasmon resonance. characterization of the sample was conducted using UV-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), and energy-dispersive X-ray analysis (EDX). The size of CuO NPs was evaluated, and their effectiveness in inhibiting biofilm formation was tested against Pseudomonas aeruginosa. Results: The UV-Vis and color change confirmed the formation of CuO NPs, with sizes ranging from 25 to 40 nm. Characterization techniques such as FTIR, XRD, SEM, HR-TEM, and EDX validated the structural and compositional properties of the nanoparticles. CuO NPs showed promising results in inhibiting biofilm formation by Pseudomonas aeruginosa. Conclusion: The study demonstrates that Hibiscus rosa-sinensis is an effective green method for synthesizing CuO NPs. The produced nanoparticles exhibit significant antimicrobial properties, particularly in preventing biofilm formation by Pseudomonas aeruginosa, highlighting their potential for biomedical and environmental applications.

Keywords: Copper oxide nanoparticle, Hibiscus rosa-Sinensis, Characterization, Antibiofilm activity.

1. Introduction

Nanotechnology has become a prominent and advanced technology in several sectors, including material science, physics, biomedical science, chemistry, medicine, cosmetics, drug delivery, environmental health, and gene delivery [1-2]. Moreover, it has been increasingly used for treating cancer, diabetes, infection, and allergic inflammation. Nanoparticles (NPs) were synthesized using various techniques, including solid-state reactions, chemical reactions, coprecipitation, and sol-gel approach. Chemically synthesized NPs include reactive functional groups with the potential to be harmful to biological systems. Therefore, environmentally acceptable techniques for producing clean, biocompatible, nontoxic, and eco-friendly substances are highly commendable. Biological approaches are considered secure, economical, sustainable, and eco-friendly. Additionally, they are suitable for food and medical applications[3-5].

In this green chemistry method, the plant extract is used as a reducing and capping agent for CuO NP synthesis because of the reducing properties of the leaf extract. CuO is a semiconductor material of the p-type, characterized by a narrow band gap of 1.2eV [6]. CuO NPs exhibit greater durability, stability, and an extended storage period than organic antibacterial agents [7-10]. It has widespread application in various fields, including catalysis, optics, solar energy, gas sensing, high-temperature superconductivity, hydrogen storage, and medicine [11-17]. The synthesis of CuO NPs can be achieved using several methods, such as sol-gel, thermal, electrochemical, precipitation, ultrasonic mixing, self-assembly, microwave irradiation, solution combustion, and hydrothermal approaches in the presence of PEG [18]. These approaches have harmful medical consequences. Therefore, it is difficult to use natural substances that are fast, gentle, convenient, and non-toxic to synthesize metal and metal oxide nanoparticles in a water-based setting. Nanoparticles, minuscule particles, can be synthesized using microorganisms and vegetation. Nevertheless, the process of synthesizing NPs from plants is highly reliable, allowing for the attainment of NPs of various sizes and forms.

Hibiscus rosa-sinensis is a plant species, is frequently known as chinese hibiscus, rose mallow, china rose, or shoe flower. It is a member of the Malvaceae family and is indigenous to East Asia. Infusion of leaves, roots, and fruits is beneficial for treating arthritis, boils, and cough. Additionally, the fruit can be applied externally to alleviate sprains, wounds, and ulcers. Hibiscus tea is rich in vitamin C. To the best of our knowledge, researchers have utilized a biological method including the use of leaf extract from H. rosa-sinensis to serve as both a stabilizing and surface-reducing agent in the production of CuO NPs. The objective of this study was to synthesized and analyze Cuo NPs using extracts from H. rosa-sinensis leaves and assess their effectiveness in preventing biofilm formation.

2. Materials and methods

2.1. Leaf extract preparation

Leaves of H. rosa-sinensis shrub were collected from Coimbatore Gardens. The leaves were washed multiple times with distilled water to eliminate contaminants, followed by a 2-day drying period at room temperature. The desiccated leaves were pulverized into a fine powder. Cu ions were converted into CuO NPs by mixing 5g of coarsely crushed leaves with 100 mL of sterilized deionized water. The mixture was filtered using Whatman filter paper. The samples were stored in a refrigerated place for future research.

2.2. Synthesis of CuO NPs

The synthesis of CuO NPs involved mixing the leaf extract with freshly made 0.01M Cu_2SO_4 solution at a 1:1 ratio. Subsequently, the concoction was incubated in a dark chamber for 48 h. Subsequently, the mixture was calcinated at 400°C for 2 h. A gray powder was obtained. The sample was ground into a fine powder using a mortar and pestle to obtain a more uniform texture, which is necessary for conducting characterization investigations.

2.3. Analysis of CuO NPs

The optical characteristics were analyzed using UV-visible spectroscopy and FTIR (Fourier Transform Infrared) spectroscopy with a Shimadzu FTIR prestige 21 instrument. The morphological and chemical contents and structures of CuO nanoparticles were analyzed using SEM-EDX (Zeiss), HR-TEM, and XRD (PAN analytical: XPERT PRO) instruments.

2.4. Bacterial strain

Pseudomonas aeruginosa was used as the experimental organism isolated from the sputum of an infected person. An overnight culture containing 10^7 CFU/mL was prepared for the study.

2.5. Preparation of experimental suspension

The required quantity of NPs was dissolved in autoclaved deionized water and sonicated to ensure that the particles did not agglomerate. A serial dilution method was employed to prepare concentrations of 0.02, 0.12, 0.24, 0.5, 1.0, 1.2, 1.4, 1.6 and 2.2 µg/mL in 100 µL of deionized water. Different concentrations of CuO NPs were transferred into a microtitre plate for biofilm assay. 20 µL of the bacterial culture described above were added to each well, and the suspension was subsequently diluted to a final volume of 200 µL by adding 80 µL of sterile LB broth.

2.6. Biofilm inhibition assay

Antibiofilm activity was assessed using microtiter plates [19]. A microtiter plate prepared earlier was incubated at 37 °C for 24 h. After incubation, the liquid medium was removed, and the wells were rinsed 5 times with sterilized distilled water to eliminate free-floating bacteria. The plates were allowed to dry in the air for 30-45 minutes. The wells were then treated with 100 µL of a 1% crystal violet solution for 45 min. The wells were washed out 5 times with sterile distilled water. The liquid that had been re-solubilized was measured at an optical density of 570 nm after the addition of 95% ethanol. The Experiment was conducted in triplicate.

3. Results

3.1. Synthesis of CuO nanoparticles

Following a 2-day incubation period, the color changed from green to brown, indicating the successful synthesis of CuO NPs using the extract of H. rosa-sinensis leaves.

3.2. Characterization of CuO NPs

3.2.1. UV-Vis- Spectroscopy

The absorption spectra obtained from UV-visible spectroscopy at a wavelength of 300 nm indicate the presence of CuO NPs. The single sharp band (Figure 1) in the absorption spectra predicts the spherical nature of the CuO NPs.

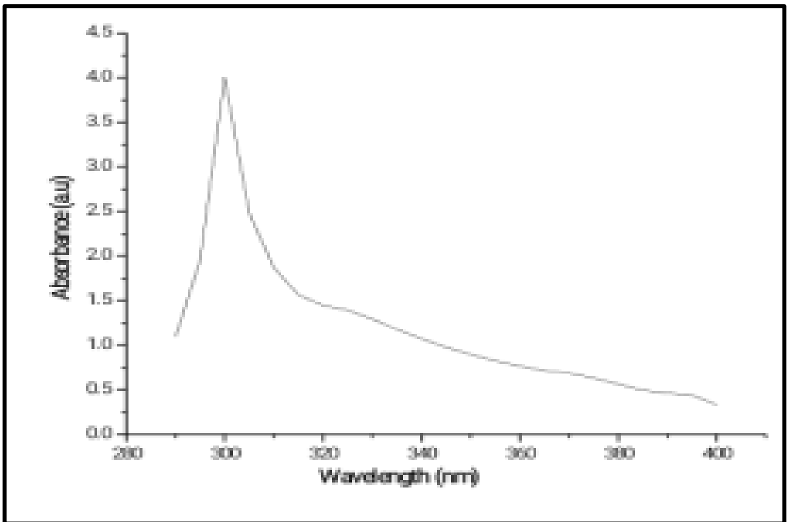

Figure 1: UV-vis-spectroscopy of copper oxide nanoparticles.

3.2.2. FTIR

FTIR spectra were recorded in the solid state using the KBr pellet technique in the range of 2000-400 cm^{-1}. Fourier transform infrared (FTIR) spectroscopy analysis of CuO nanoparticles, which were synthesized using a green method and then subjected to calcination at a temperature of 400 ºC, is presented in Figure 2. The FTIR spectra show peaks at 580, 548, and 530 cm^{-1}, which can be attributed to the vibrations of CuO. This result confirms the synthesis of highly pure CuO NPs.

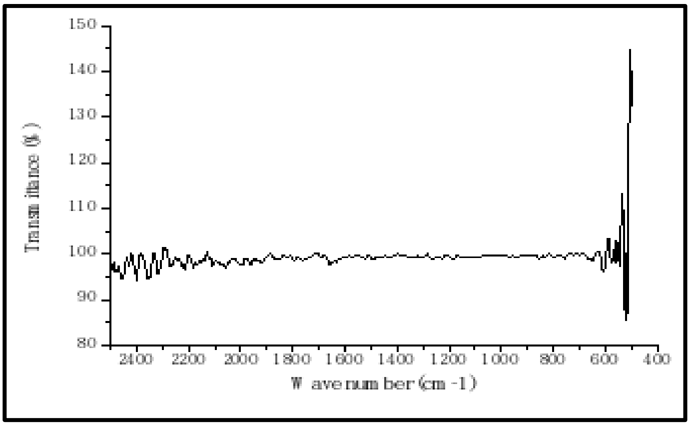

Figure 2: FTIR spectroscopy of copper oxide nanoparticles

3.2.3. XRD analysis

The powdered sample was analyzed using a Cu Kα –X-ray diffractometer to determine the presence of CuO NPs and analyze their structure. Figure 3 displays the XRD diffraction peaks at 2θ angles of 34.20°, 37.42°, 37.97°, 46.28°, 51.77°, 54.99°, 58.50°, 65.22°, and 68.13, which correspond to the (111), (111), (200), (202), (020), (021), (113), (113), and (312) planes, respectively. The XRD spectrum revealed that the CuO NPs exhibit a crystalline character and a spherical structure, which was confirmed by the International Center of Diffraction Data Card (JCPDS file no: 80-1917). The absence of any other phases indicates high-quality CuO NPs. The average dimensions of CuO NPs were obtained using the Debye-Scherrer equation [20]:

The formula $D = K\lambda / \beta \cos\theta$ represents the relationship between the mean crystalline size (D), X-ray wavelength (λ), fullwidth at half maximum of the XRD line (β), and half diffraction angle (θ). Scherrer's constant (K) was 0.94, and the X-ray wavelength was 0.1546 nm. The CuO nanoparticles had an average size of 25–40 nanometers, indicating their nanocrystalline structure.

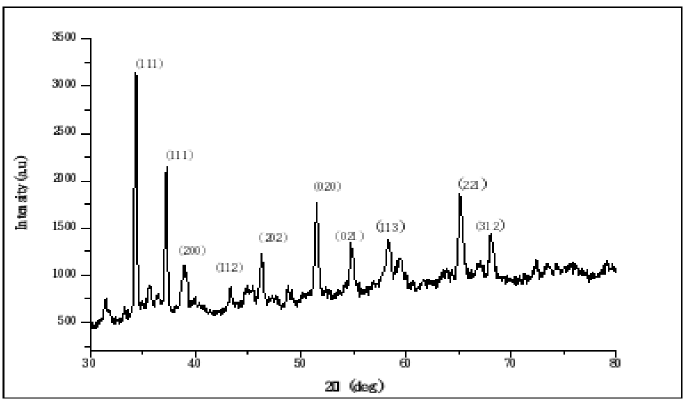

Figure 3: X-ray diffraction pattern of copper oxide nanoparticles

3.2.4. SEM and TEM analysis

The morphologies of the CuO NPs were analyzed by scanning electron microscopy (SEM). The SEM images in Figure 4 depict CuO NPs, which exhibit various shapes, including spherical, cubic, and irregular. The presence of copper (Cu) and oxide (O) peaks was confirmed by EDX analysis. The atomic percentage of copper (Cu) was determined to be 79.73%, whereas the atomic percentage of oxygen (O) was determined to be 11.40%. The HRTEM image confirmed that the CuO nanoparticle has a spherical shape (Figure 5).

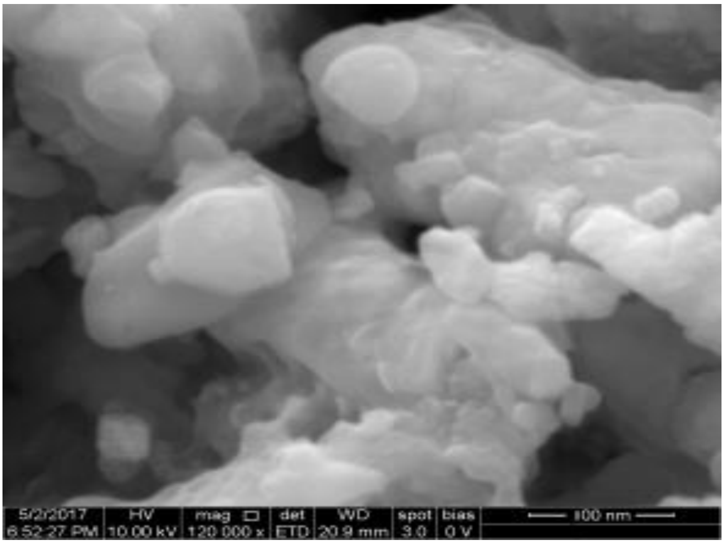

Figure 4: SEM image of copper oxide nanoparticles

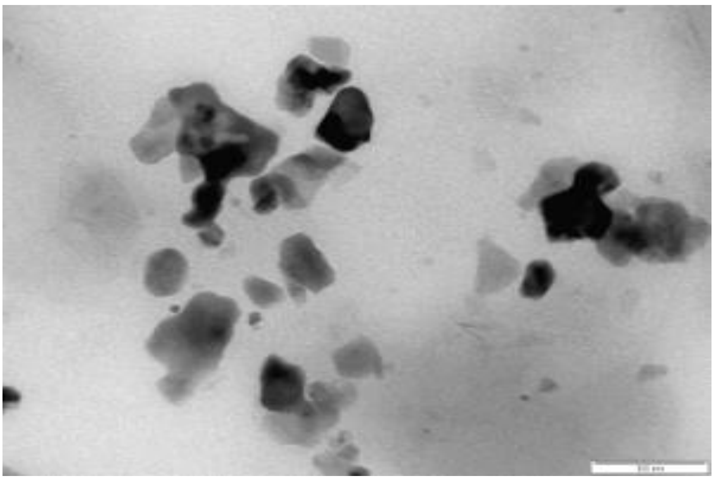

Figure 5: HR-TEM image of copper oxide nanoparticles

3.3. Antibiofilm activity of CuO NPs

The inhibition of biofilm formation by CuO NPs was observed at the lowest concentration of 0.02 µg/mL, which was approximately 93.48% (Figure 6a, 6b). Biofilm inhibition was noticed to increase with the increase in the concentration of CuO NPs. CuO NPs at concentrations above 0.5 µg/mL evaluated distinct biofilm inhibition of Pseudomonas aeruginosa. The antibiofilm activity of CuO NPs was analyzed using SEM to confirm the biofilm inhibition by CuO at the lowest and highest concentrations (Figure 7).

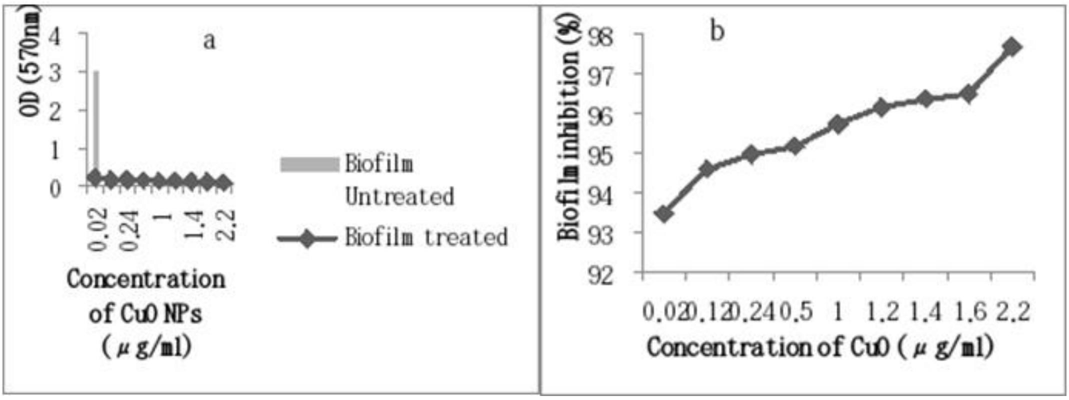

Figure 6: (a) Graph showing biofilm quantity in control and at different concentrations of copper oxide nanoparticles, and (b)Biofilm inhibition of Pseudomonas aeruginosa in the presence of copper oxide nanoparticles

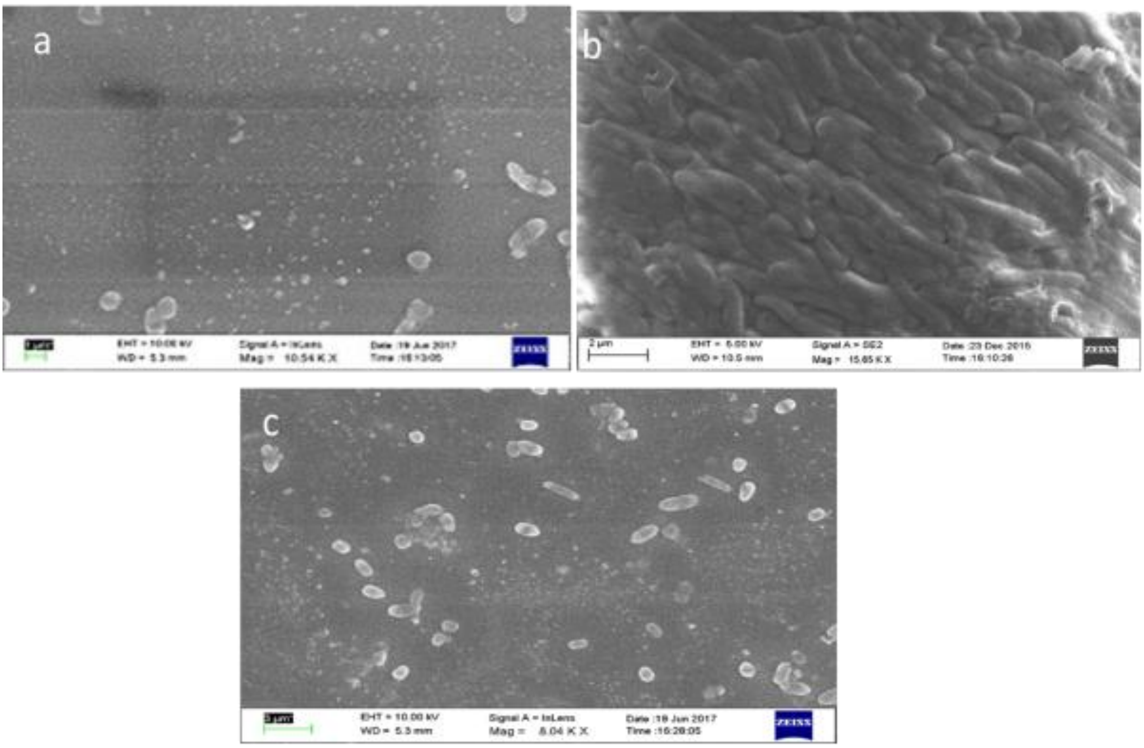

Figure 7: Fe-SEM image of antibiofilm effect of copper oxide nanoparticles (a) biofilm untreated,(b) biofilm treated with 0.02 µg/ml, and(c) biofilm treated with 2.2 µg/ml

4. Discussion

The biological synthesis of metal NPs is becoming increasingly important in health, biotechnology, chemistry, and environmental research fields. The physical and chemical methods of metal nanoparticle production were noticed for a long time; however, it was investigated that metal NPs are produced very recently. The leaf extract of H. rosa-sinensis allows for the rapid and eco-friendly synthesis of CuO NPs, providing an efficient approach for their synthesis.The use of plant extracts avoids the use of hazardous and damaging stabilizing and reducing chemicals. CuO NPs generated in this study were 25–40 nm in size.

The antibiofilm activity of CuO NPs strongly influenced the reduction of biofilm formation in Pseudomonas aeruginosa. The results showed that the inhibition of biofilm formation was enhanced by increasing the CuO NP concentration. This result indicates a clear correlation between the concentration of CuO NPs and their inhibitory effect on biofilm formation, demonstrating a dose-dependent action. It seems that the first target site of copper damage is the cell wall of bacteria. It has been suggested that in some microorganisms, copper oxidative damage might occur through the Fenton mechanism [21]. However, this effect was possible only with the treatment of high concentrations of CuO NPs. CuO NPs have high adsorption capacity, absorption capacity, and penetration, making them an efficient antibiofilm agent.

Copper is the most essential cofactor of many enzymes, but high levels of copper are toxic. Bacteria must find an appropriate balance to prevent toxicity [22]. The results showed that a concentration of 0.02 µg/mL inhibited biofilm formation by 93.48%. Biofilm formation of Pseudomonas aeruginosa was completely suppressed at the highest CuO NP dose (2.2 µg/mL), resulting in a 97.68% inhibition rate.

5. Conclusion

In this study, we demonstrate the sustainable synthesis of CuO NPs using the leaf extract of Hibiscus rosa-sinensis. The successful formation of CuO NPs was confirmed by a color change from green to brown resulting from surface-plasmon resonance. Comprehensive characterization using UV-vis spectroscopy, FTIR, XRD, SEM, HR-TEM, and EDX analysis revealed the spherical and crystalline nature of the CuO NPs, ranging in size from 25 to 40 nm. CuO NPs synthesized biologically exhibited significant antibiofilm activity against Pseudomonas aeruginosa. Notably, they effectively inhibited the growth of bacteria at a minimal concentration of 0.02 µg/mL. The antibiofilm efficacy increased at higher concentrations, demonstrating over 93% inhibition at the lowest tested concentration and further enhanced inhibition above 0.5 µg/mL. The use of H. rosa-sinensis leaf extract as a reducing and stabilizing agent offers a sustainable and nontoxic approach to nanoparticle synthesis, aligning with the principles of green chemistry. The results of this study underscore the potential of plant-based methods to produce biocompatible nanoparticles for various biomedical applications, especially in combating biofilm-associated infections. These promising results encourage further exploration of plant extracts in nanoparticle synthesis for diverse applications in medicine and environmental health.

Declaration of interest

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

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References

1. Yi, L., Fan, H., Yuan, S., Li, R., Wang, H., Quan, Y., ... & Wang, Y. (2024). Antimicrobial Resistance and Biofilm Formation of Bordetella bronchiseptica in Central China, with Evidence of a Rare Heteroresistance Strain to Gentamicin. Animals, 14(9), 1301.
2. Anjum, S., Ishaque, S., Fatima, H., Farooq, W., Hano, C., Abbasi, B. H., & Anjum, I. (2021). Emerging applications of nanotechnology in healthcare systems: Grand challenges and perspectives. Pharmaceuticals, 14(8), 707.
3. Baranwal, J., Barse, B., Fais, A., Delogu, G. L., & Kumar, A. (2022). Biopolymer: A sustainable material for food and medical applications. Polymers, 14(5), 983.
4. Ali, N. J., & Hassan, K. H. (2023). Green synthesis of zinc oxide nanoparticles using leaves extract of capparis for adsorption of nickel (II) and copper (II) ions. In AIP Conference Proceedings, 2475(1).
5. Demirel, Ö., Güneş, H., & Can, C. (2024). Sustainable and modern bio-based technologies: new approachs to food safety and security. Environment, Development and Sustainability, 1-28.
6. Tadjarodi, A., & Roshani, R. (2014). A green synthesis of copper oxide nanoparticles by mechanochemical method. Current Chemistry Letters, 3(4), 215-220.
7. Rakhshani, A. E. (1986). Preparation, characteristics and photovoltaic properties of cuprous oxide—a review. Solid-State Electronics, 29(1), 7-17.
8. Premkumar, T., & Geckeler, K. E. (2006). Nanosized CuO particles via a supramolecular strategy. Small, 2(5), 616-620.
9. Ren, G., Hu, D., Cheng, E. W., Vargas-Reus, M. A., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. International journal of antimicrobial agents, 33(6), 587-590.
10. Zhang, Z., Che, H., Wang, Y., Song, L., Zhong, Z., & Su, F. (2012). Preparation of hierarchical dandelion-like CuO microspheres with enhanced catalytic performance for dimethyldichlorosilane synthesis. Catalysis Science & Technology, 2(9), 1953-1960.
11. Rahnama, A., & Gharagozlou, M. (2012). Preparation and properties of semiconductor CuO nanoparticles via a simple precipitation method at different reaction temperatures. Optical and Quantum Electronics, 44, 313-322.
12. Chen, G., Zhou, H., Ma, W., Zhang, D., Qiu, G., & Liu, X. (2011). Microwave-assisted synthesis and electrochemical properties of urchin-like CuO micro-crystals. Solid state sciences, 13(12), 2137-2141.
13. Yu, Y., & Zhang, J. (2009). Solution-phase synthesis of rose-like CuO. Materials Letters, 63(21), 1840-1843.
14. Uvarajan, K. P., and K. Usha. "Implement A System For Crop Selection And Yield Prediction Using Random Forest Algorithm." International Journal of communication and computer Technologies 12.1 (2024): 21-26.
15. Yu, T., Cheong, F. C., & Sow, C. H. (2004). The manipulation and assembly of CuO nanorods with line optical tweezers. Nanotechnology, 15(12), 1732.
16. Zhu, J., Bi, H., Wang, Y., Wang, X., Yang, X., & Lu, L. (2007). Synthesis of flower-like CuO nanostructures via a simple hydrolysis route. Materials Letters, 61(30), 5236-5238.

17. Safarifard, V., & Morsali, A. (2012). Sonochemical syntheses of a nano-sized copper (II) supramolecule as a precursor for the synthesis of copper (II) oxide nanoparticles. Ultrasonics sonochemistry, 19(4), 823-829.
18. Nagajyothi, P. C., Muthuraman, P., Sreekanth, T. V. M., Kim, D. H., & Shim, J. (2017). Green synthesis: in-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells. Arabian journal of chemistry, 10(2), 215-225.
19. Cao, M., Wang, Y., Guo, C., Qi, Y., Hu, C., & Wang, E. (2004). A simple route towards CuO nanowires and nanorods. Journal of nanoscience and nanotechnology, 4(7), 824-828.
20. Djordjevic, D., Wiedmann, M., & McLandsborough, L. A. (2002). Microtiter plate assay for assessment of Listeria monocytogenes biofilm formation. Applied and environmental microbiology, 68(6), 2950-2958.
21. Jacobs, P. A., Derouane, E. G., & Weitkamp, J. (1981). Evidence for X-ray-amorphous zeolites. Journal of the Chemical Society, Chemical Communications, (12), 591-593.
22. Cide, Felip, José Urebe, and Andrés Revera."Exploring Monopulse Feed Antennas for Low Earth Orbit Satellite Communication: Design, Advantages, and Applications." National Journal of Antennas and Propagation 4.2 (2022): 20-27.
23. Borkow, G., & Gabbay, J. (2009). Copper, an ancient remedy returning to fight microbial, fungal and viral infections. Current Chemical Biology, 3(3), 272-278.
24. Vaishampayan, A., De Jong, A., Wight, D. J., Kok, J., & Grohmann, E. (2018). A novel antimicrobial coating represses biofilm and virulence-related genes in methicillin-resistant Staphylococcus aureus. Frontiers in Microbiology, 9, 221.