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BRIDGING THE POWER OF THE OCEAN AND NANOTECHNOLOGY: HARNESSING *Porphyra Indica* AND *Gracilaria Salicornia* SEAWEEDS TO SYNTHESIZE ECO-FRIENDLY COPPER OXIDE NANOPARTICLES

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ABSTRACT

The immense, untapped potential of the ocean offers an innovative pathway for sustainable nanotechnology, where natural resources like the seaweeds *Porphyra indica* and *Gracilaria salicornia* play a crucial role in the eco-friendly synthesis of copper oxide nanoparticles. "UV spectrum reveals that the peaks at 448 nm and 564 nm correspond to the CuO NPs' absorption. FTIR spectrum showed the presence of alcohols, amine, and phenolic groups that are mainly accountable for producing CuO-NPs. SEM analysis revealed that the average size of the synthesised CuO nanoparticles was between 14.8 and 15 nanometres. EDAX spectrum showed pure CuO-NPs as only copper and oxygen related peaks are present without any impurity peaks, confirming the formation of CuO-NPs. X-ray diffraction (XRD) studies revealed the monocyclic crystalline phase of CuO-NPs. The good reduction behaviour from Cyclic Voltametric studies confirms the formation of copper oxide. CuO-NPs demonstrate significant antibacterial efficacy, exhibiting inhibition zones larger than 11 mm against five different bacterial strains. This highlights the dual advantage of using *Porphyra indica* and *Gracilaria salicornia* not only in sustainable nanoparticle synthesis but also in advancing antimicrobial applications.

Keywords: *Porphyra Indica*, *Gracilaria Salicornia,* SEM Analysis, EDAX Spectrum, XRD Studies, Antibacterial Activity, Electrochemical Analysis, Life Below Water.

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INTRODUCTION

The development and alarming rise of "multi-drug-resistant (MDR) bacteria" has put antibiotic effectiveness in danger and created a major threat to community health throughout the biosphere. Many bacterial diseases are growing more resistant to antibiotics, and treatment failures with antibiotics have led to thousands of fatalities. Nanomaterials' amazing optical, physical, chemical, and mechanical qualities have led to their unavoidable use in recent years. Notably, due to their numerous uses in pharmacological activities, CuO-NPs have captured the attention of every researcher. Copper oxide nanoparticles are also gaining popularity on behalf of their cheap and abundant in contrast to gold and silver metals and also their capable potential for use in many fields due to their availability and low cost compared to gold and silver metals, as well as their potential for use in a wide range of sectors, copper oxide nanoparticles are likewise becoming more and more popular. CuO-NPs have taken the position of nano-silver in the treatment of wastewater and as antibacterial agents. Due to their availability and low cost compared to gold and silver metals, as well as their potential for use in a wide range of sectors, copper oxide nanoparticles are likewise becoming more and more popular. CuO-NPs have taken the position of nano-silver in the treatment of wastewater and as antibacterial agents.[1] At present, nanoscale metals are mainly synthesised by chemical methods that have unintended effects such as environmental pollution, large energy consumption, and potential health problems. In response to these challenges, green synthesis, which uses seaweed extracts instead of industrial chemical agents to reduce metal ions, has been developed.

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Green synthesis is more beneficial than traditional chemical synthesis because it costs less, decreases pollution, and improves environmental and human health safety.[2] Researchers have recently focused on natural antioxidants, especially those derived from macroalgae. The vibrant red colouration of red seaweed, which belongs to the Rhodophyta group of macroalgae, is caused by pigments like PE phycoerythrin (PE) and phycocyanin (PC). These pigments give red seaweed its unique look and play a major role in its bioactive qualities, which include antibacterial, anti-inflammatory, and antioxidant activities. The vibrant red colouration of red seaweed, which belongs to the Rhodophyta group of macroalgae, is caused by pigments like PE and PC. These pigments give red seaweed its unique look and play a major role in its bioactive qualities, which include antibacterial, anti-inflammatory, and antioxidant activities.[3] *Gracilaria* is a genus of macroalgae that may serve as a source of antioxidant chemicals for the development of chemotherapeutic medicines. This potential is also supported by its nutritional contents, so that residents in several countries on the Asian continent, especially in Indonesia.[4] It is encouraged that more research effort is put into the modification and optimisation of green synthesis techniques to help improve the NPs yield. The objective of this paper is to report the synthesis and characterize Copper nanoparticles CuO-Nps using two different seaweeds *Porphyra indica* and *Gracilaria salicornia,* and their applications.

EXPERIMENTAL

Porphyra indica and *Gracilaria Salicornia* seaweeds were collected from the coastal area, Rameswaram, India. To remove epiphytes, salt, sand, microorganisms, and other suspended materials associated with seaweeds, fresh seaweeds were rinsed using sterile seawater.

Preparation of Seaweed Samples for Experimental Studies

The collected whole seaweeds were uniformly cut into small fragments and shade-dried. The dried seaweeds were added to 5 ml of sterile seawater, and again they were dried. The sample materials were granulated or powdered with a blender and sieved to produce homogeneous particles using sieve No. 60. The final homogeneous seaweed powder was employed in a variety of experimental tests. Dried seaweed powder (10g) was extracted with ethanol, heated on the hot plate for one hour, and after cooling extract was filtered. The supernatant solution is to be used for further analysis.

Sustainable Synthesis

Copper oxide nanoparticles (CuO-NPs) were obtained by combining *Porphyra indica* and *Gracilaria salicornia* seaweeds extract in a 1:9 ratio with a 1 mm concentrated anhydrous copper sulphate solution.[5] The prepared mixture was stirred at room temperature for two hours. CuO nanoparticles were synthesised (Fig.-1), as demonstrated by the solution's changed brown colour. After 24 h of incubation, the colloidal solution was centrifuged at 10,000 rpm for 10 min to obtain a pellet, which was then purified by washing three to five times with double-distilled water. The final residue obtained was placed in a muffle furnace at 400 °C to eliminate the linked substance that is organic substance from seaweeds; the solid product was filtered and then dried at room temperature. The powder was mashed in a mortar-pestle so as to get a fine nature of sample for further characterization by using SEM, EDAX, UV, XRD, and FT-IR Spectroscopy.

Characterisation of Copper Oxide Nanoparticles

A UV-visible spectrophotometer was used to measure the absorbance of transition metal ions and organic substances at a specific wavelength. The copper oxide nanoparticles were scanned inside using a Perkin-Elmer spectrophotometer in a range of wavelengths starting from 380 to 800 nm, and the absorption spectrum was also detected for the sample. Fourier Transforms Infra-Red (FTIR) analysis was used to detect the presence of a functional group that surrounds the nanoparticle.[6] The FTIR characterisation peaks in the range of 400–4000 cm^{-1}. Scanning Electron Microscopy (Jeol JSM-6480 LV SEM) at the voltage of 10–20 kV was employed to determine the size and solid surface morphology of the copper oxide nanoparticles. For determining the structure of synthesised nanoparticles, XRD (PAN analytical X'pert Pro) of scattering angle (2h) between 20 and 80 degrees was taken for the dried powder. Energy-dispersive X-ray spectroscopic analysis (EDAX) was used to detect the compositional studies of CuO-NPs.

Electrochemical Studies

Cyclic voltammetry is a technique for electrochemical experiments with a potential range of -1 V to 1.2 V, was carried out. The sample used maximum peak current used was 200 μA. Metronhm Drop

Sense screen-printed electrode (SPE) and supporting electrolyte as CV utilised 0.1 mm KCl/10 mm (Fe(CN)6) 3-/4- at a pH of around 7.0.

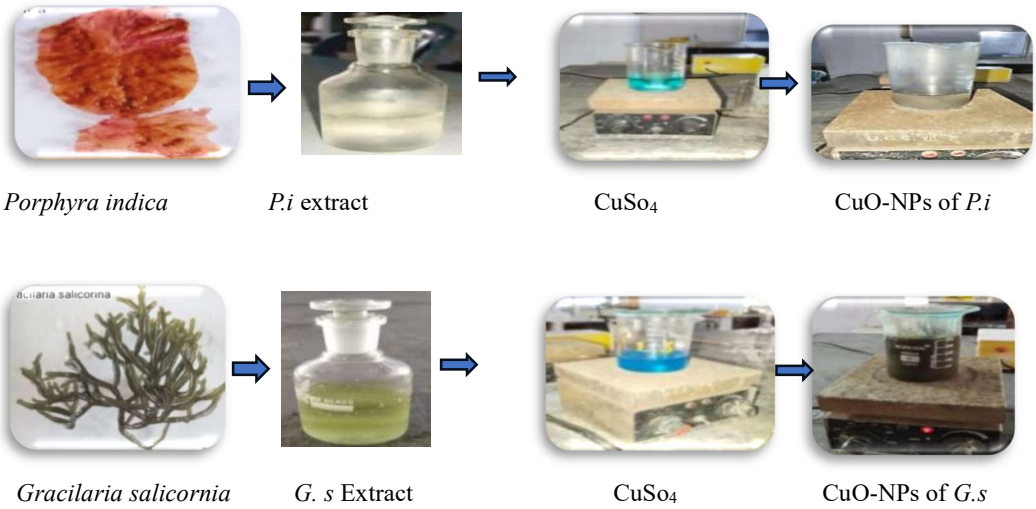

Fig.-1: The Synthesis of Copper Oxide Nanoparticles using a *Porphyra indica* and *Gracilaria salicornia* Seaweeds

All electrochemical characterisations were done at room temperature only. The SPE was changed using the drop dry approach. About 2 mg of as-prepared CuO NPs were distributed in a tiny amount of DMF to create a paste, which was then sonicated. A small amount of paste was dropped in the middle of the SPE working electrode. The carbon tube had a diameter of 4 mm and a volume of 50 µL. It included a silver reference electrode and a carbon counter electrode. The SPE was dried at room temperature and used in electrochemical investigations.[7]

Antibacterial Efficiency

Antibacterial activities of synthesised CuO-NPs were effective a *Staphylococcus aureus(S. aureus), Bacillus cereus (B. cereus), bacteria Escherichia coli (E. coli), Bacillus subtilis (B.subtilis),*and *Pseudomonas aeruginosa (P.aeruginosa)*were found using the Muller-Hinton agar well method. It is also used to examine several types of seaweed extracts and their corresponding CuO-NPs.[8]*Streptomycin* was utilised as a positive control. After solidification, 0.1 mL of a 3 to 4 hrsat 35 ºCold culture of bacteria tested was placed on the medium and spread throughout the plates of the entire surface. Then, using a sterile cork borer, a hole with a diameter of 6 to 8 mm is punched. A volume of 1000 µg/ml of the extract solution the desired concentration, is injected into the well. The agar plates are then incubated at 37 degrees Celsius for 18 to 24 hours. The extract diffuses throughout the agar medium, inhibiting the growth of the microbial strain tested. After the incubation period, a clear zone around the well was measured, and the stated inhibitory zone diameter in mm was stated, which is the area between two corners of the zone.[9]

RESULTS AND DISCUSSION

Optical-Spectroscopy Studies

UV-vis absorption measurement provided primary evidence for the production of CuO-NPs. UV-vis absorption spectra can be used to determine the structural properties of nanoparticles. Fig-2 depicts the UV-vis absorption spectra and band gap energy of the generated CuO-NPs. Fig-2 (a) and (b) show absorption as a function of wavelength. An absorbance peak was observed in the ultraviolet wavelength range. A distinctive absorption peak occurred at 448 and 564 nm, which corresponded to the previously described absorption band of CuO-NPs.[10,11] The observed characteristic absorption peak indicates the formation of CuO nanoparticles. Fig-3 shows the FTIR spectra of the chemical synthesis of CuO-NPs, where samples were scanned in the 500–4000 cm^{-1} frequency range. The stretching of the amine (N-H) and phenol (O-H) groups is responsible for the band at 3000–3350cm^{-1}. Absorption peaks attributed to aromatic C-H bending[11] are located between 820 and 880 cm^{-1}.The vibrational frequencies of CuO[12] are represented by the absorption bands found at 600–700cm^{-1}. The C-O stretching and bending of C-H[13] is linked to the absorption band at 1118 cm^{-1} and 1400 cm^{-1}. As a result, the FTIR spectrum revealed the presence of phenolic, amine, and alcohol groups, which are

primarily responsible for the green method's production of CuO-NPs. These groups also serve as reducing and capping agents and prevent agglomeration.

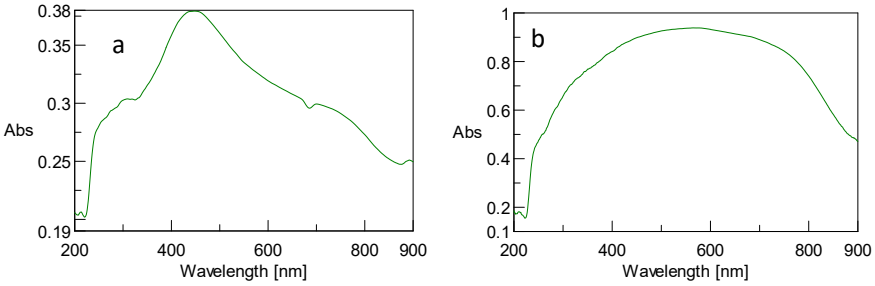

Fig.-2: (a) and (b): The CH-CuO nanoparticles' UV-visible Spectra Demonstrate Absorbance at 448 nm for P.i.Extract and 564 nm for G.s.Extract. CH-CuO Nanoparticle Characterisation

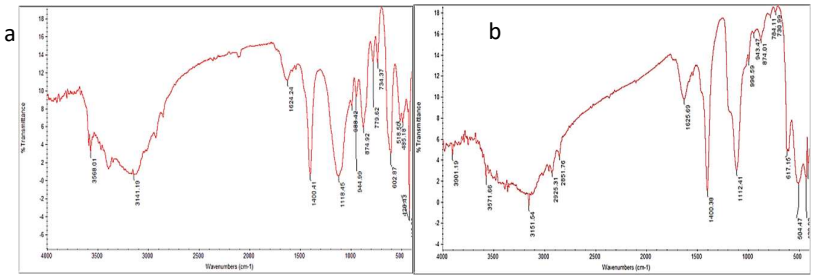

Fig.-3: (a) and (b): FTIR Analysis for the CuO Nanoparticles Showing Peaks at Various Ranges

Morphological and Elemental Analysis

SEM micrographs of prepared CuO-NPs are displayed in Fig.-4. To optimise the morphology of fabricated CuO-NPs, the extract concentration of *P.indica* and *G. Salicornia* seaweeds was varied. The SEM micrographs of NPs synthesised by 30 mL and 50 mL of seaweeds extract are depicted in Fig.-4(a) and (b), respectively. From the SEM micrographs, it is clear that CuO-NPs are spherical-like in shape with an average size range of 14.8-15 mm. The elemental composition and purity of synthesised CuO-NPs were explored using EDAX, and the spectrum is given in Fig.-5. The EDAX spectrum showed pure CuO-NPs as only copper and oxygen-related peaks are present without any impurity peaks.

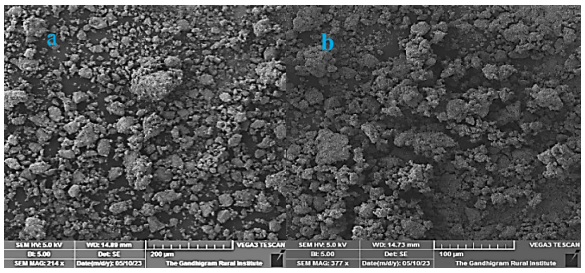

Fig.-4 (a) and (b): SEM Images of CuO-NPs using *P. indica* and *G.Salicornia*

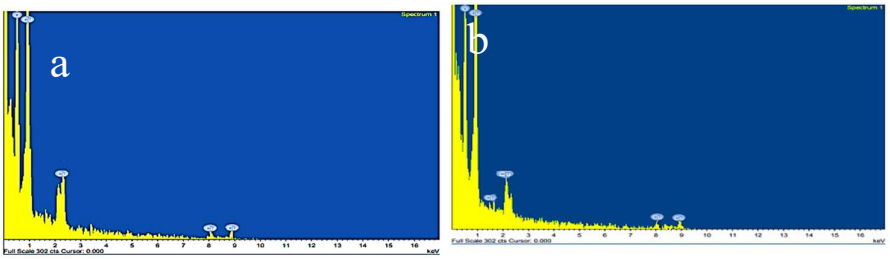

Fig.-5: (a)and (b): EDAX Image of Cu and O Element Percentage

Structural Analysis

XRD investigation revealed the crystalline structure of CuO-NPs, as shown in Fig.-6(a) & (b), with diffraction peaks found at the 2θ values of29.1,31.98,33.70, 35.50, 37.65, 46.04, 59.40, 67.83, 72.82, and 73.99 were in accordance to the (110), (002), (111), (200), (021), (102), (120), (012), (201), and

(210) planes, respectively, which confirmed that CuO–NPs were synthesised successfully. CuO–NPs crystallised in a monoclinic system with the space group of C2/c, and their peaks were detected at scattering angles of 2θ (JCPDS card No. 89–5899).[14,15] Debye-Scherer was used to estimate the size of these particles.[16]

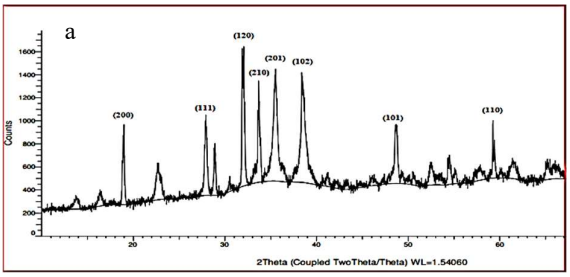

Fig.-6 (a): XRD Patterns of CuO-NPs Synthesised using Copper Sulphate and *P.indica*Extract

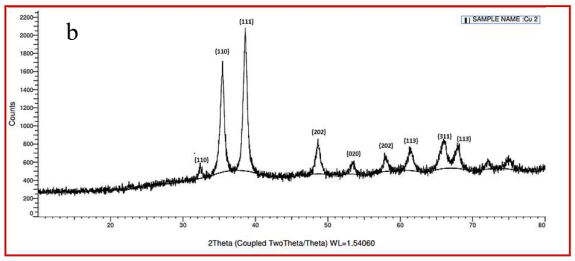

Fig.-6 (b): XRD Patterns of CuO-NPs Synthesised using Copper Sulphate and *G.salicornia*Extract

Applications of CuO-NPS

CuO-NPs synthesised with seaweed extracts have been shown to have a wide range of uses in various industries. Chemical and Pharmaceutical applications of CuO-NPs were discussed below, as shown in Fig.-7 and 8.

Electrochemical Analysis

Cyclic voltammograms of CuO-NPs were recorded in the potential range from -0.40 to 1.2 V, Syn CuO-NPs coated on a glassy carbon electrode (GCE), Ag/AgCl, and platinum wire were used as working, reference, and counter electrode, respectively. Fig-7 shows the cyclic voltammogram of iron oxide at different scan rates of 25mV, 50mV, 75mV and 100mV at produced different cathodic peaks was obtained. The voltammogram shows the reduction peaks at 879 mV and 737 mV, respectively. The good reduction behaviour confirms the formation of copper oxide.

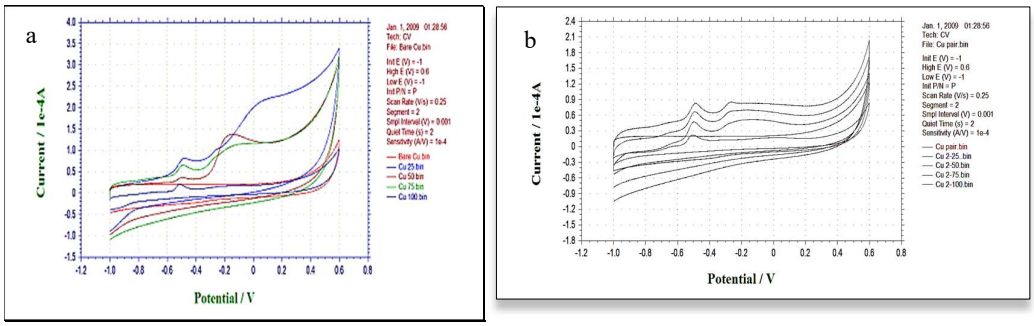

Fig.-7 :(a) and (b): Cyclic Voltametric Behaviour of Copper Oxide Nanoparticles at Different Scan Rates.

Pharmaceutical Activity

The antimicrobial potential of CuO nanoparticles synthesised via a green method using *P.indica* and *G.salicornia* seaweed was investigated. These nanoparticles against two human pathogenic microorganisms were assessed, namely *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Bacillus subtilis and Pseudomonas aeruginosaas* shown in Table-1 and Fig.-8. In the beginning, at smaller quantities, the nanoparticles indicated an average antibacterial activity with a zone of inhibition.

However, as the concentration of the CuO nanoparticles increased, the antibacterial activity against bacteria significantly improved. The positive control drug, Ampicillin, displayed the highest zone of inhibition, with 15 mm against the one organism, respectively. Interestingly, the green-synthesised *P.indica* and *G.salicornia* seaweeds of CuO-NPs nanoparticles demonstrated greater antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*, demonstrating their greater ability to combat bacteria. The spherical form and size of the nanoparticles, which range from 2 to 20 nm, are probably responsible for some of their increased biological activity. Overall, the results suggest that *P.indica* and *G.salicornia* seaweeds-mediated CuO-NPs nanoparticles synthesized via a green method possess potent antibacterial properties against five different bacteria, with a zone of inhibition greater than 11 mm.

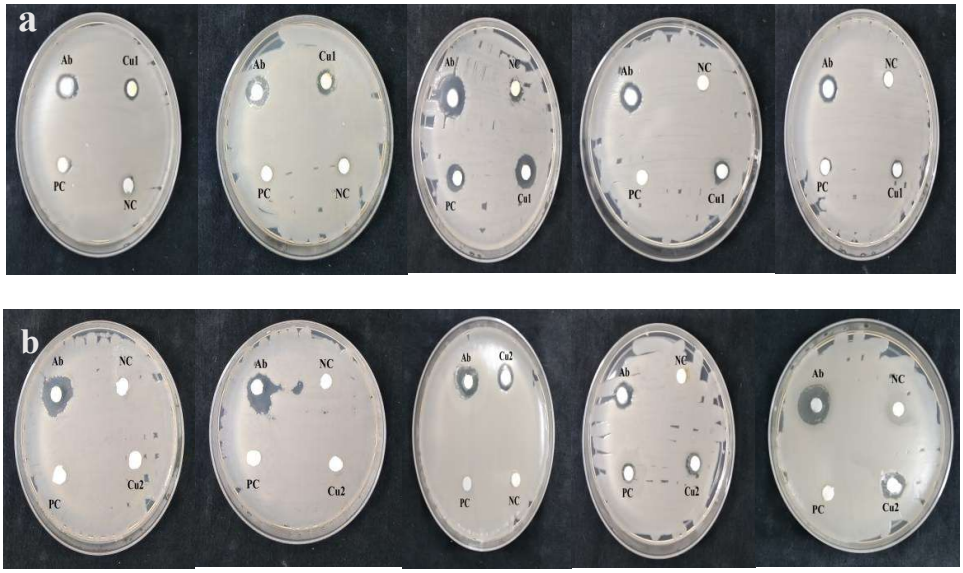

Fig.-8: (a) and (b): Antibacterial activity of *P.indica and G.salicornia* extract using CuO-NPs against *(S.aureus, B.cereus, E.coli, B. subtilis, and P.aeruginosa)*

Table-1: Antibacterial Activity (Zone of inhibition) of *P.indica* and *G.salicornia* Seaweeds-Mediated CuO-NPs

Bacteria	Inhibition zone in mm							
	Ampicillin, Ab	Positive control, PC	Negative control, NC	P.i Nps of CuO	Ampicillin, Ab	Positive control, PC	Negative control, NC	G.s Nps of CuO
E.coli	9	-	-	6	15	-	-	-
Staphylococcus aureus	10	-	-	7	10	-	-	-
Bacillus subtilis	15	10	-	11	11	-	-	7
Bacillus cereus	8.5	-	-	6	14	6	-	8
Pseudomonas aeruginosa	8	-	-	5	10	-	-	11

CONCLUSION

A natural, eco-friendly green synthesis technique was employed in this study, demonstrating that seaweed extracts from *Porphyra indica* and *Gracilaria salicornia* can effectively induce the formation of copper oxide nanoparticles (CuO-NPs). Scanning electron microscopy (SEM) analysis revealed that the synthesised CuO-NPs were predominantly spherical, with sizes ranging from 2 to 200 nm. X-ray diffraction (XRD) analysis confirmed that the CuO-NPs exhibited a monoclinic crystalline phase. Furthermore, Fourier-transform infrared (FTIR) spectroscopy identified functional groups such as alcohols, amines, and phenolics, which play a crucial role in the green synthesis process. The biosynthesised CuO-NPs showed significant concentration-dependent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Additionally, under typical visible light irradiation, these nanoparticles exhibited promising photocatalytic activity by degrading the crystal violet (CV) cationic dye. Overall, the study concludes that green photo-synthesised CuO-NPs are potent antibacterial agents with environmentally benign photocatalytic

properties, underscoring their potential for sustainable applications in biomedicine and environmental remediation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-14: Life Below Water*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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