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J. Mukila

Manonmaniam sundaranar University

S. Sankaravadivu

sankaravadivuresearch@gmail.com

Manonmaniam sundaranar University

Research Article

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Posted Date: February 25th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8890559/v1>

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Additional Declarations: No competing interests reported.

Biosynthesized CuO nanoparticles mediated by red algae *Gracilaria textorii*: Photocatalytic dye degradation of methylene blue and in vitro antioxidant and antidiabetic activities

J. Mukila¹, S. Sankaravadivu^{1*}

¹Research Scholar (Reg-No 23112012032001),

^{1,*}PG & Research Department of Chemistry, A.P.C Mahalaxmi College for women, Thoothukudi, Affiliated to Manonmaniam sundaranar University, Abishekapatti, Tirunelveli-627012, Tamilnadu, India.

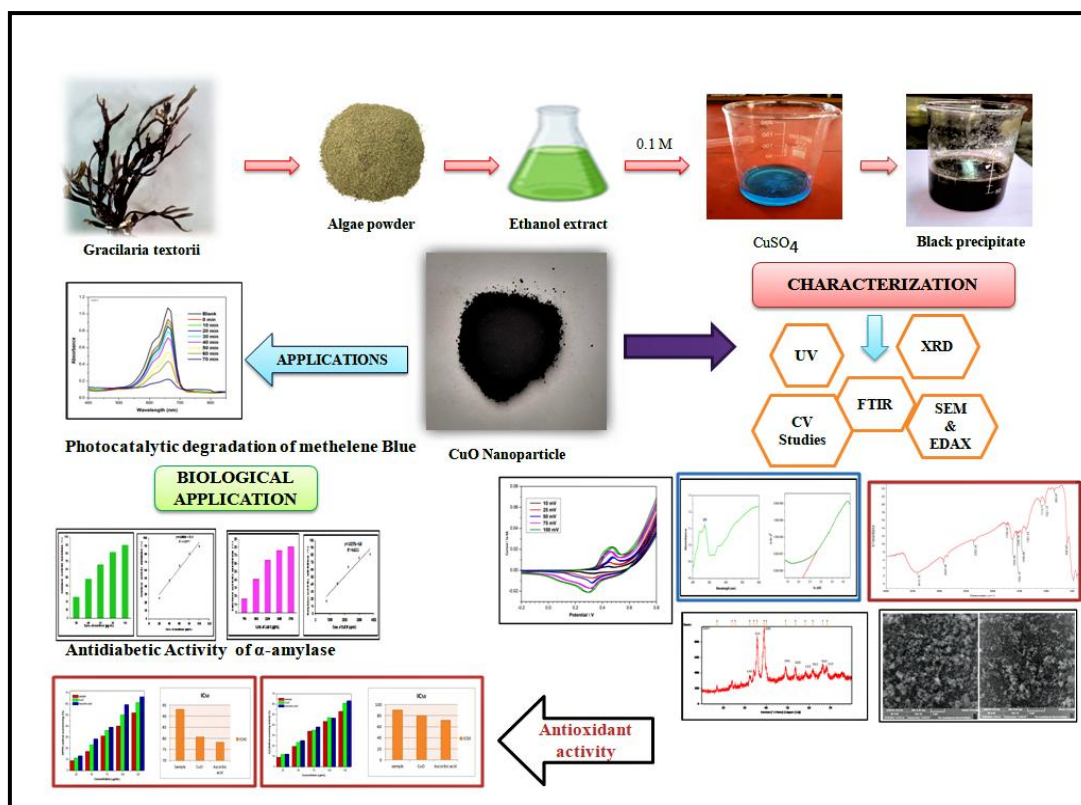
Corresponding Author: S.Sankaravadivu (sankaravadivuresearch@gmail.com)

Abstract:

In this Current Study, copper oxide (CuO NPs) was synthesized by an eco-friendly, low-cost, and economical synthesis method using *Gracilaria textorii* algae extract as a reducing and capping agent. The synthesized CuO NPs were characterized by UV–visible spectroscopy showed an absorption edge at 272 nm, and the optical band gap was estimated to be 2.2 eV, confirming the nanoparticles. The FTIR spectrum shows that the band in the range 400–4000 cm^{−1} confirms the formation of CuO NPs. X-ray diffraction patterns revealed the monoclinic phase of CuO with good crystallinity, and the average crystallite size was calculated as 17.8 nm. The SEM analysis also confirmed that formed nanoparticles had irregular and agglomeration morphology and Elemental analysis and the particle size were confirmed by elemental dispersive X-ray analysis (EDX) and particle size analyzer using confirm the Cu and O nanoparticles. Electrochemical behavior was investigated through cyclic voltammetry, which indicates good redox activity and electron transfer capability of the synthesized CuO NPs. The photocatalytic activities of biosynthetic CuO NPs and chemically prepared CuO NPs were studied using methylene blue upon exposure to sunlight irradiation revealing significant degradation efficiency, with variation between the algae - mediated nanoparticles. Moreover, Antioxidant activity was evaluated using DPPH and hydrogen peroxide (H₂O₂) scavenging assays, indicating concentration-dependent radical scavenging showed strong antioxidant activity. In vitro study showed that significantly inhibited the enzymatic activity of-amylase inhibition assay. The biosynthesized copper oxide nanoparticles method are environment and economically use in various field and biomedical applications.

Keywords: Antidiabetic, Antioxidant, Biomedical, Copper Oxide, Economical, *Gracilaria textorii*, Photocatalytic

GRAPHICAL ABSTRACT:



INTRODUCTION:

Copper oxides have been gaining interest as nanomaterials due to their unique optical, thermal, electrical, chemical, and biological properties. [1, 2] Copper oxide nanoparticles are cheap and abundant in contrast to gold and silver metals and they also have potential application in many fields. [3] CuO nanoparticles, which include vapor deposition to electrochemical reduction, thermal decomposition, and chemical reduction. [4-7] Generally, nanoparticle synthesis methods are grouped into physical, chemical, and biological approaches [8]. Because of its unique properties, copper oxide nanoparticles are now being used in technology and medicine such as biomedical [9] Recently, several researchers had embarked on the assessment of the biological activities of metal oxide nanoparticles, such as copper oxide, from which improved biological and photocatalytic activities better than those obtained from metal nanoparticles have been reported. [10]

Marine algae represent some of the earliest and most diverse life forms in oceanic ecosystems. They range from microscopic phytoplankton to large multicellular seaweeds, exhibiting significant diversity in morphology and function. Based on pigmentation and cellular organization, algae are broadly classified into three groups: green algae (Chlorophyta), brown algae (Phaeophyceae), and red algae (Rhodophyta) [11,12]. Marine algae are recognized as rich reservoirs of bioactive constituents such as polyphenols, flavonoids, polysaccharides, carotenoids, proteins, amino acids, alkaloids, sterols, and terpenoids. These bioactive compounds contribute to their antioxidant, antibacterial, and antifungal properties, highlighting their importance in biomedical research [13]. Red algae, belonging to the division Rhodophyta, are widely consumed in many countries due to their distinctive flavor and high nutritional value, including essential vitamins and proteins [14].

The genus *Gracilaria textorii* consists of intertidal and subtidal red seaweeds, comprising nearly 130 species that are widely distributed, particularly in subtropical regions, and are available throughout the year [15]. Biological synthesis of nanoparticles using plant extracts and organisms such as bacteria, algae, and fungi has emerged as an eco-friendly and sustainable alternative to conventional methods. In this Study a simple, cost-effective, and green synthesis of CuO-NPs using *Gracilaria textorii* algae extract using UV-visible spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and field emission scanning electron microscopy with energy dispersive x-ray spectroscopy (FESEM-EDX) analysis, the structural properties, and electrochemical studies of the algae extract. This study demonstrates that marine algae can serve as efficient, inexpensive, and green sources for the synthesis of copper oxide nanoparticles. Furthermore, the photocatalytic degradation of the methylene blue (MB) dye, antioxidant and antidiabetic activities of CuO-NPs were established evidently. The synthesized CuO nanoparticles, thus, proved to be an interesting material for electrochemical and biological application studies.

2. Materials and methods:

2.1 Collection of seaweed:

Seaweed samples were obtained from the shallow coastal waters of the Gulf of Mannar (Latitude 9°17'N, Longitude 79°17'E) at Mandapam, on the southeast coast of India. The collected seaweeds were washed with salt water to remove all the epiphytes and then allowed in distilled water for about 15 min to remove the salts. Thus, the collected seaweeds were air - dried, then cut into small pieces ground into powder, and used for further studies. [16]



Fig.1 *Gracilaria textorii*

2.2 MATERIALS:

2.2.1 Preparation of copper Sulphate: Copper Sulphate (CuSO_4) analytical grade was purchased from Merck & Co. and used without further purification. Double distilled demonized (DI) water was used throughout the course of this investigation. Whatmann No 1 filter paper was used for filtration. A solution of CuSO_4 was prepared by dissolving the solid CuSO_4 in DI water.

2.3 Preparation of Algae Extract

The Collected red algae Polythene bags were used to carry the samples to the laboratory and rinsed in freshwater to remove dust, debris, and salt content. Then the algae were again cleaned using a brush to remove the epiphytes, dust, and attached organisms and washed twice with distilled water. The cleaned algae were dried at room temperature in the shade for a week. The dried algae were ground into a fine powder using a mechanical grinder and stored in container box and further use preparation of extract. [17,18] The collected extracts were evaporated by a rotary evaporator under vacuum (50°C) and stored at 4 °C for further use.

2.4 Preparation of algae extract: Red algae dried seaweed powder (10 gm) was soaked in 200 ml ethanol (95%) and shaken at room temperature for 24h. Then, Whatman paper No. 1 was used to filter the mixture and the extracts were evaporated to dryness in a rotary evaporator at 45°C. Stock solutions of algae extract were prepared for further studies. [19]

2.5 Biosynthesis of Copper oxide Nanoparticles:

For synthesis of CuO nanoparticles, 0.1M copper sulphate solution was dissolved in 100 ml of distilled water. 50 ml of seaweed ethanol extract was added to 50 ml of copper sulphate solution and mix well. The pH of the solution was controlled by 1M NaOH, fixing the values at 5, 7, and 10 with continued stirring. [20] After that, the green color changed to a black precipitate, and the solution was centrifuged and dried in an air oven at 60 °C for 24 h. The well-dried copper oxide nanoparticles were stored in an airtight bottle for further characterization.

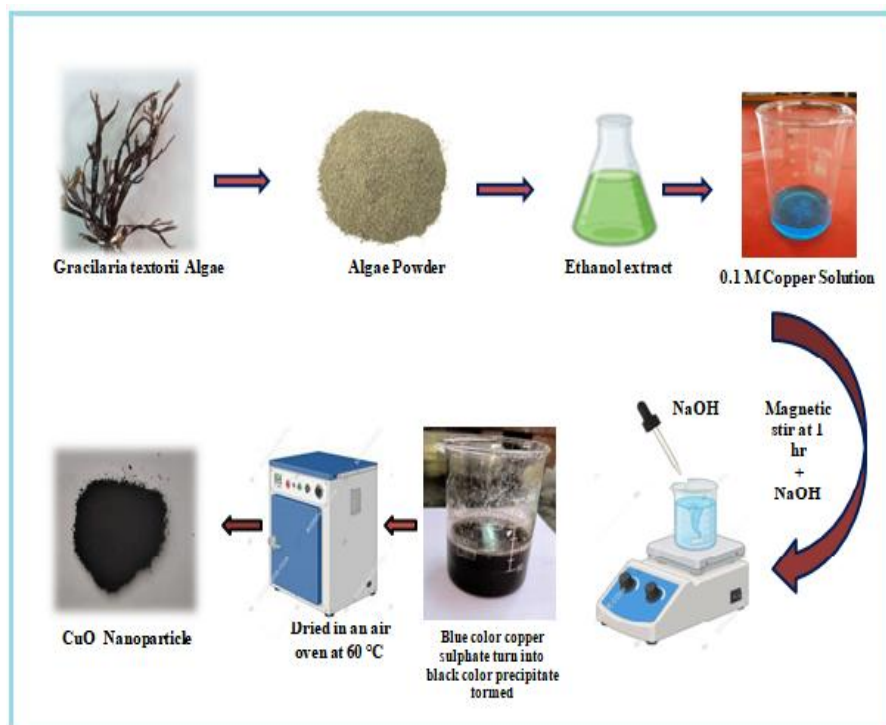


Fig 2: Schematic Diagram of the synthesis of CuO NPs using *Gracilaria textorii*

3. CHARACTERISATION:

UV–Visible spectral analysis was carried out using a JASCO V-650 UV–Visible spectrophotometer. X-ray diffraction (XRD) analysis was performed with an analytical X'Pert Powder X'Celerator diffractometer over a 2θ range of 10° to 80°, and the crystallite size was determined using the Scherrer equation. Fourier-transform infrared (FT-IR) spectra were obtained with a Nicolet iS5 instrument. The surface characteristics of the synthesized nanoparticles were examined using a JEOL JSM-6700F scanning electron microscope, along with SEM and EDAX measurements for morphological and elemental analysis. Electrochemical investigations were conducted using a CH Instruments Electrochemical Workstation (Model 650c).

3.1 Photocatalytic Activity of Methylene Blue:

The photocatalytic activity of the CuO was assessed using methylene blue (MB). The experiment was carried out in an open atmosphere under the sunlight irradiation. Take 100 ml of MB solution and 5 mg of CuO NPs sunlight irradiation. The suspension was mixed using magnetic stirring in the dark for 30 min to establish adsorption and desorption equilibrium. The suspension sample was collected and filtered to separate solid particles at regular time intervals.[21] At 10 min intervals, the catalyst was separated by centrifugation, and the supernatant absorbance was recorded at 664 nm in the methylene blue and examined using a JASCO V-650 UV Visible spectrophotometer.

3.2 Antioxidant Activity of Synthesized CuO Nanoparticles

3.2.1 DPPH radical scavenging activity:

The free radical scavenging activity of the synthesized nanoparticles copper oxide on the stable radical 2,2-diphenyl-2-picrylhydrazyl (DPPH) was estimated [22,23]. Synthesized nanoparticles algae extracts at different concentrations (12.5, 25, 50, 100 and 200 µg/mL) were incubated with 3.0 mL of a DPPH methanol solution (0.1 mM) in dark for 30 min at room temperature followed by measuring the absorbance at 517 nm using UV-Vis spectrophotometer (Genesys 10s UV: Thermo electron corporation). Ascorbic acid was taken as the control standard. The percentage of DPPH radical inhibition was using the formula given below.

$$\% \text{ DPPH radical scavenging activity} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 is the absorbance of the control

A_1 is the absorbance of the test samples and reference.

All the tested samples were performed in triplicates and the results were averaged.

3.2.2 Hydrogen peroxide radial scavenging activity

As per the method [24,25] Hydrogen peroxide (H_2O_2) scavenging ability of the synthesized nanoparticles ascorbic acid was established. In phosphate buffer (pH 7.4) a solution of H_2O_2 (40mM) was arranged. Dissimilar

concentrations (12.5, 25, 50, 100 and 200 µg/mL) of nanoparticles ascorbic acid in 3.4 ml phosphate buffer were added. These are added to H₂O₂ solution (0.6 ml, 40 mM). At 230nm, the absorbance value of the reaction mixture was recorded. The scavenging % of H₂O₂ was calculated using the formula given below.

$$\text{Scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Here A₀ is the absorbance of control and A₁ is the absorbance of the sample

3.3 In vitro antidiabetic activity:

Alpha amylase assay

The enzyme assay mixture (800 µl) consists of 125 µg/ml of porcine α-amylase, 1% of the starch, and 50 mM potassium phosphate buffer (pH 7). The reaction mixture was incubated at 25 °C for 1 h and terminated by the addition of 1 ml of the DNS reagent [3, 5 dinitro salicylic acid (1%), Na-K tartrate (12%) in 0.4 M NaOH]. The reaction mixture was put in a bath of boiling water for 15 min and then cooled down to room temperature. The α-amylase activity was determined at 540 nm using an Epoch 2 multi-well plate reader (Biotek, USA) [26].

Inhibition of alpha amylase enzyme activity by compound

For the inhibitory study, a total reaction volume of 700 µL was prepared containing porcine α-amylase (125 µg/mL), different concentrations of the test sample, and 50 mM potassium phosphate buffer maintained at pH 7. This reaction mixture was kept at room temperature for 30 minutes. Subsequently, 1% starch solution was introduced and allowed to react for an additional 30 minutes under the same conditions. Following incubation, the activity of the α-amylase enzyme was assessed as previously described.

4. RESULT AND DISCUSSION

4.1 UV-Visible spectrophotometric analysis:

The CuO NPs were investigated with UV–Visible spectroscopy to identify the optical band gap shown in the Fig.3a UV–visible spectra of the aqueous extract of *Gracilaria textorii* were observed in the presence of copper oxide nanoparticles at 200–800 nm. The absorption peak displayed a sharp peak at 272 nm, which corresponds to the surface plasmon resonance (SPR) band characteristic of CuO nanoparticles. This observation confirms the formation of CuO NPs, as similar peaks have been reported in earlier studies of biogenic and chemically synthesized CuO nanoparticles. [27] The absorption in the UV region can be attributed to the intrinsic electronic transitions of CuO associated with charge transfer from the O²⁻ (2p) valence band to the Cu²⁺ (3d) conduction band. The relatively narrow and intense peak suggests the formation of small and uniformly distributed nanoparticles. [28]

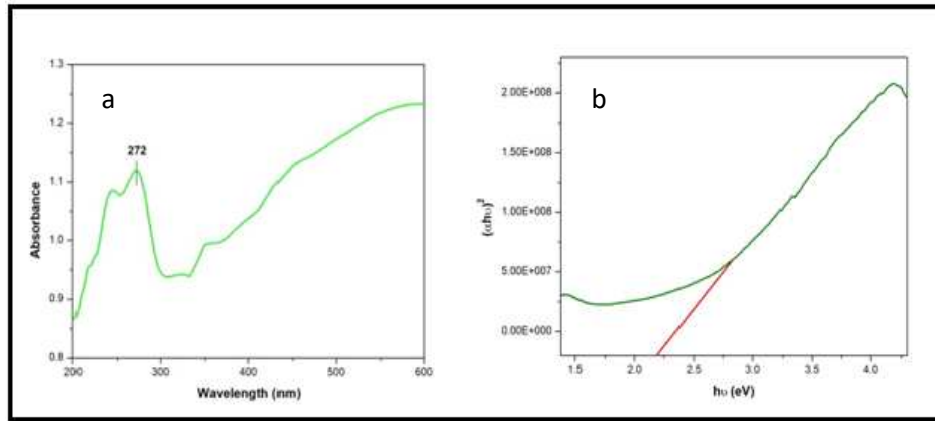


Fig. 3 a) UV-Vis spectroscopy Absorption and b) Tauc plot of CuONPs using *Gracilaria textorii*

The optical band gap energy was estimated using the Tauc relation equation.

$$(\alpha h\nu)^2 = A (h\nu - E_g) \quad \dots\dots (1)$$

Where $h\nu$ is the light energy, α is the absorption coefficient, E_g is the direct band gap, k is a constant. [29] The optical band gap was determined using the Tauc relation, and the extrapolated value was found to be 2.2 eV (Fig 3 b). The observed band gap is slightly lower than that of bulk CuO (~ 2.7 eV), which may be due to quantum confinement and surface defect effects [30, 31]. This reduced band gap improves visible-light absorption and makes the nanoparticles suitable for photocatalytic and electrochemical applications.

4.2 Fourier Transform Infrared (FTIR)

The FTIR technique was realized in the range of $400\text{--}4000\text{ cm}^{-1}$ in order to understand the role played by the biosynthesis of CuO NPs using the algae extract of *Gracilaria textorii* Fig.4 shows The broad peak in the region $3500\text{--}3000\text{ cm}^{-1}$ can be seen as the result of multiple absorptions, such as those due to OH, NH, and CH stretching. [32] The peak at 2923.39 cm^{-1} represents C–H stretching Vibration of aliphatic compounds.[33] The band at 1636.4 cm^{-1} and 1458.88 cm^{-1} are associated with O–H bending and C=C aromatic ring stretching Vibration. [34, 35] which also play a role in capping the nanoparticles. The absorption bands at 1381.63 cm^{-1} and 1114 cm^{-1} may be assigned to C–N and C–O stretching vibrations of amine and alcohol groups. A Peak around 1021.13 cm^{-1} is attributed to the –C–O bond of polyols and carbohydrates such as flavonoids, polysaccharides, terpenoids, etc. of algae [36] The absorption band at 608 cm^{-1} [37] corresponds to the stretching vibration of the Cu–O bond in monoclinic confirming the formation of copper oxide.

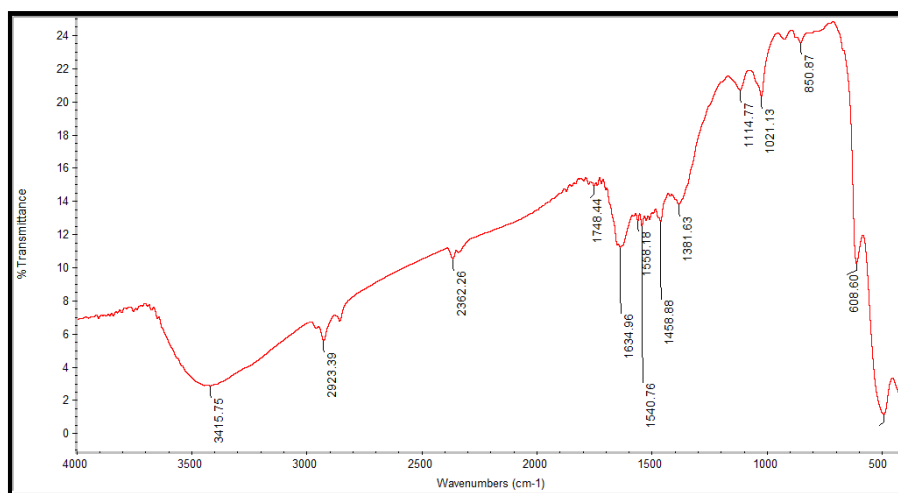


Fig. 4 FTIR analyses of copper oxide nanoparticles using *Gracilaria textorii*

4.3 X-Ray Diffraction (XRD):

The X-ray diffraction (XRD) pattern of the synthesized CuO nanoparticles using extract of *Gracilaria textorii* is shown in **Fig.5**. The diffraction peaks at 2θ values of approximately 32.3786, 35.8016, 49.0775, 53.6419, 58.2917, 61.8215, which are assigned to the (110), (111), (200), (202), (113), (311) planes of monoclinic CuO NPs with the standard JCPDS card no. 80-191650 [38]. The average size was determined by Debye Scherer's Eq. (2) [39]

$$D = \frac{K \lambda}{\beta \cos \theta} \quad \dots\dots\dots (2)$$

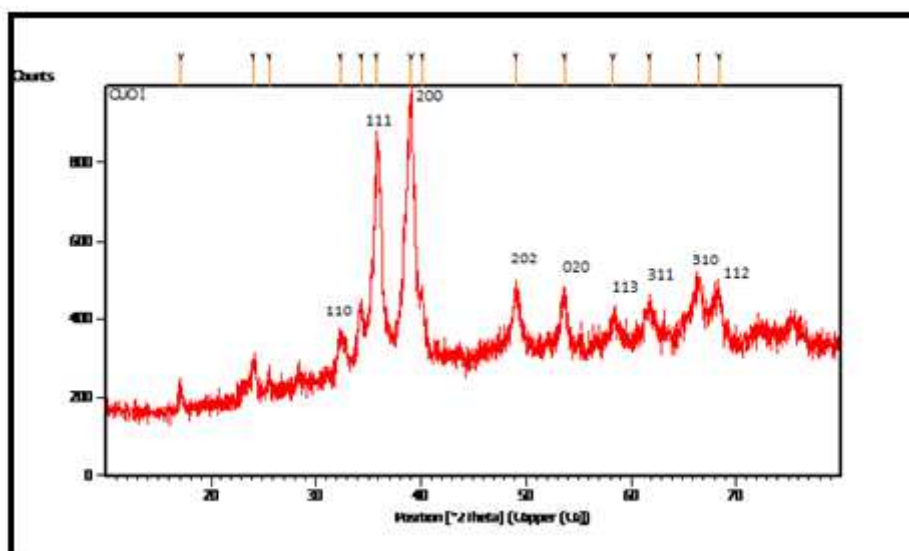


Fig. 5 XRD patterns of copper oxide nanoparticles of algae extract

Here, D represents the crystallite size in Ångströms, λ is the wavelength of the X-rays used (1.54 Å), k is a constant taken as 0.9, β is the full width at half maximum (FWHM) of the diffraction peak, and θ denotes the Bragg angle. The average crystallite size of the nanoparticles was calculated using the Debye–Scherer equation, considering multiple diffraction peaks to obtain a total average rather than a single plane. The calculated average crystallite size is approximately 17.8 nm. [40]

4.4 Scanning Electron Microscopy (SEM) Analysis:

The surface morphology of the biosynthesized copper oxide nanoparticles was examined by using *Gracilaria textorii*. The images showed that the particles are mostly irregular in shape with some rod and agglomeration shapes is seen in the Fig.6 SEM analysis confirmed the formation of copper oxide nanoparticles. The SEM micrograph shows, the size of the obtained CuO NPs was in the 2 μm size range.

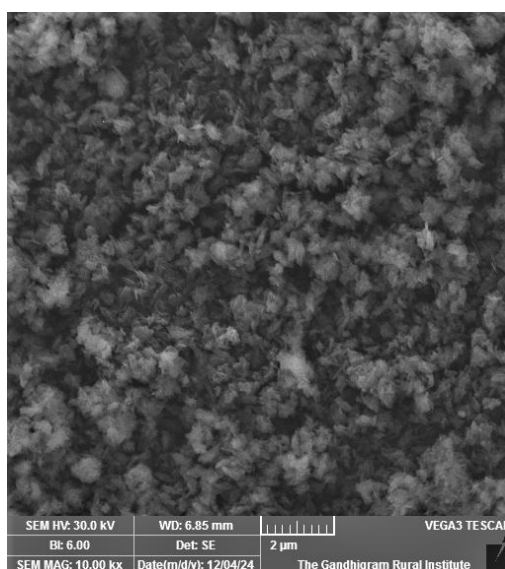


Fig. 6 SEM images copper nanoparticle synthesized by *Gracilaria textorii* magnification size 2 μm

4.5 Energy Dispersive X-Ray Spectroscopy (EDAX):

Fig. 7 The elemental composition of the algae extract was analyzed by EDX analysis. The EDX analysis reported clearly shows the presence of the Cu and O atoms present in the nanoparticles. The presence of carbon and oxygen in the NPs originated from the bioactive chemical constituents present in the aqueous extract of *Gracilaria textorii*. The atomic % of copper 37.37% was found to be low. Oxygen 62.63% was high and was detected in the EDX spectra confirming the copper oxide nanoparticles.

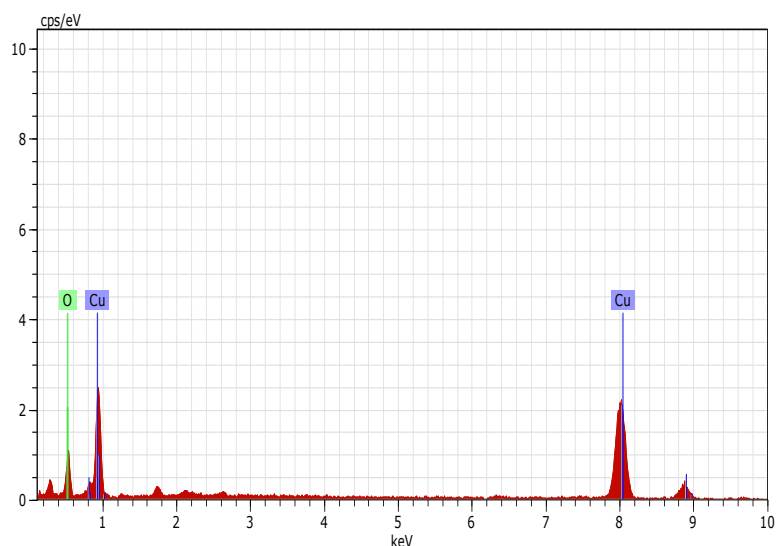


Fig.7 EDX spectrums for copper oxide nanoparticle using marine algae extract

4.6 Electrochemical Study using Cyclic Voltammetry:

Cyclic voltammetry (CV) in KOH (1M) solution was used to observe electrochemical characteristics of electrodes coated with CuO nanoparticles.[41,42] The standard three-electrode system seen in electrochemical workstations consists of a working electrode (glassy carbon), a counter electrode (Pt wire), and a reference electrode (Silver-silver chloride). Using a scan rate of 10-100 mVs⁻¹ The cyclic voltammetry (CV) analysis of the Synthesized CuO nanoparticles was carried out at different scan rates ranging from 10 to 100 mV/s within the potential window of -0.2 V to +0.8 V Fig. 8 This result shows clear anodic and cathodic peaks corresponding to the redox transition of CuO, with is attributed to the Cu²⁺/ Cu⁺ redox couple. The sharp and reproducible peaks indicate efficient electron-transfer kinetics, confirming the electroactive nature of the nanoparticles modified electrode.[43] The shape is a quasi – rectangular, with oxidation and reduction peaks observed at 0.45 to 0.55 V. The peak current increased with increasing scan rate, indicating surface-controlled electrochemical activity and good electron transfer ability. [44]

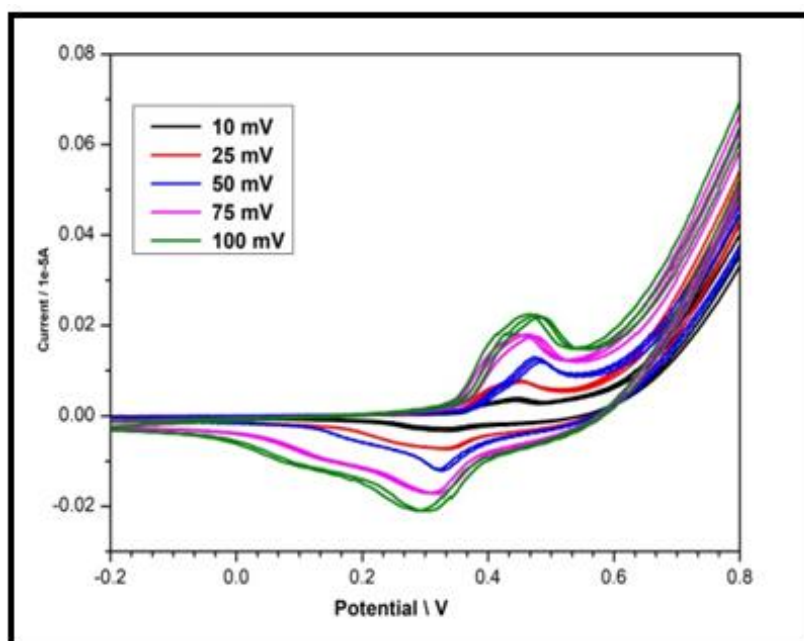


Fig. 8 cyclic voltammetric behavior of CuO oxides 1M KOH at different scan rates 10,25,50,75 and 100 mV/s.

The Plot Peak current vs scan rate analysis (Fig. 9) demonstrates that both anodic and cathodic peak currents increase linearly with scan rate, confirming that the electrochemical process is mainly governed by surface-controlled reactions. The nanosized CuO offers a high density of electroactive sites along with an enlarged surface area, which facilitates fast electron transfer. This electrochemical behavior agrees well with earlier studies on CuO-based electrodes that reported similar scan-rate-dependent characteristics [45]. Although peak widening at elevated scan rates indicates a minor diffusion-controlled contribution, the dominant current response clearly arises from surface redox processes. The improved electrochemical performance is primarily due to the large surface area, reduced crystallite size, and the presence of Cu–O bonds in the synthesized CuO nanoparticles [46].

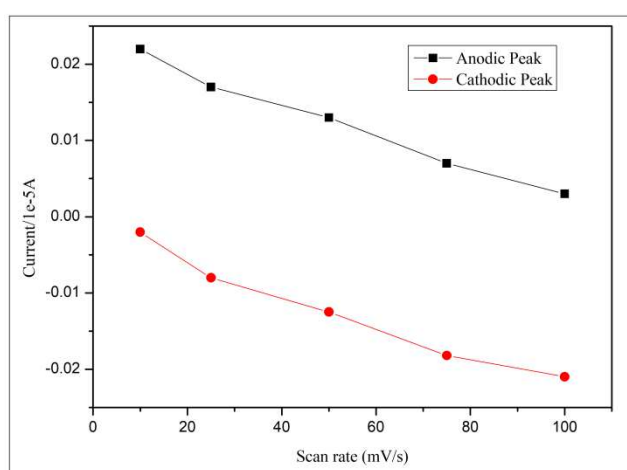


Fig. 9 cyclic voltammograms for the CuO-Nps plot of current vs. scan rate

The anodic current decreases slightly from ~0.02 mA to ~0.005 mA, while the cathodic peak becomes more negative with increasing scan rate. The corresponding correlation coefficient both anodic and cathodic peaks was $R^2 = 0.9959$ and 0.9684 . This peaks current increase linearly with scan rate. This clear dependence of current on scan rate reinforces that the synthesized CuO nanoparticles are highly electro active and suitable for electrochemical sensing and catalytic applications.

5. APPLICATIONS:

5.1 Photocatalytic Activity of Methylene Blue:

The photocatalytic activity of CuO nanoparticles was evaluated using methylene blue (MB) dye under sunlight irradiation in the presence of synthesized nanoparticles. The UV-Vis absorption spectra in Fig.9 show a strong absorption peak of MB appearing at 664 nm, corresponding to the characteristic peak of methylene blue. As the irradiation time increased from 0 to 70 min, the absorbance intensity of methylene blue gradually decreased, confirming the degradation of the dye.

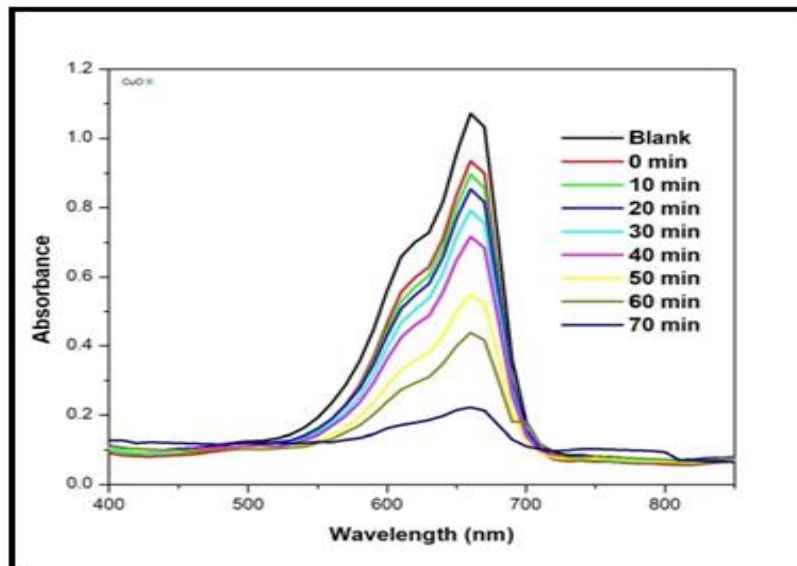


Fig. 10 UV-Vis absorption spectra of photocatalytic degradation of methylene blue using seaweed extract

The percentage of dye degradation is calculated according to equation (3)

$$\% \text{ Degradation} = \frac{(C_0 - C_t)}{C_0} \times 100 \quad \dots\dots (3)$$

Where C_0 = initial concentration of dye before exposure to visible light irradiation,

C_t = concentration of dye at a specific time t

Fig. 10 The photocatalytic study revealed that CuO nanoparticles using algae extract were capable of degrading methylene blue dye under visible light irradiation. However, *Gracilaria textorii* exhibited higher degradation efficiency (76%) suggesting better photocatalytic activity. The decrease in absorbance intensity with time

clearly demonstrates the breakdown of dye molecules. This confirms the photodegradation of methylene blue solutions with CuO nanoparticles.

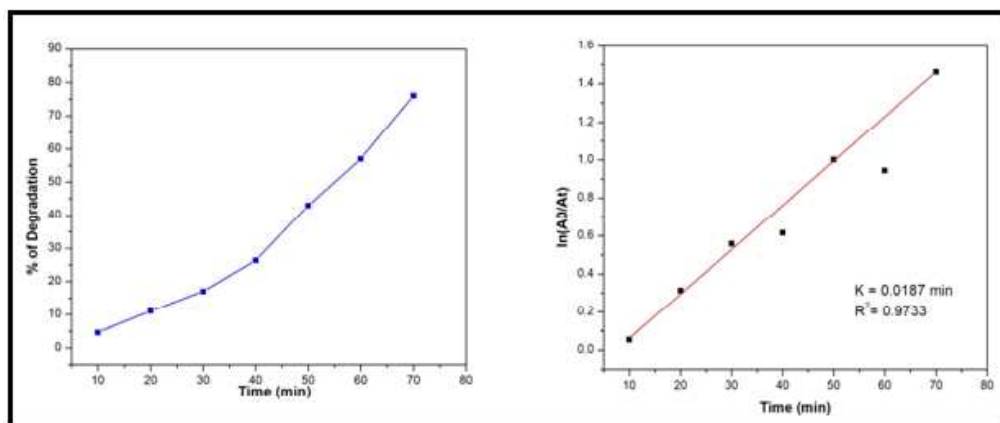


Fig.11 Percentage of degradation and Plot of $\ln(A_0/A_t)$ versus time for the degradation of MB CuO-NPs

The first-order kinetic model, can be expressed as equation (4)

$$\ln(A_0/A_t) = \ln(C_0/C_t) = -kt \quad \dots\dots (4)$$

Where, A_0 and A_t are the ratio of the absorbance of MB at times = 0 and t , respectively, C_0 and C_t are the initial concentrations, k is the first-order rate constant for the reaction (min^{-1}), and t is the response time in minutes.[47] Therefore, the rate constant (k) for MB dye degradation by synthesized CuO-NPs was calculated by the pseudo first - order equation. The evolution of versus time showed good linear correlation with the values of coefficient (R^2) determined to be 0.9733. Then, the slope of the fitting line determined the rate constant values 0.0187 min^{-1} . This result showed the excitation of the sunlight, the phenol and flavonoid groups present in the extract promoted the transfer of electrons to the conduction band of CuO, which enhances the conversion of oxygen to superoxide radicals and thus accelerates the photodegradation of biosynthesized CuO-NPs catalysts. [48]

5.2 Antioxidant Activity:

5.2.1 DPPH Radical Scavenging Activity:

The antioxidant activity of copper oxide nanoparticle synthesized from *Gracilaria textorii* algae extract was determined using the DPPH assay is shown in Fig.12 The antioxidant activity of the algae extract, CuO nanoparticles, and ascorbic acid (standard) was evaluated using the DPPH free radical scavenging method at different concentrations (25–125 $\mu\text{g/mL}$). DPPH activity showed a dose-dependent increase in radical scavenging activity. The IC_{50} values were found to be 90.08 $\mu\text{g/mL}$ for algae extract, 82.16 $\mu\text{g/mL}$ for CuO nanoparticles, and 78.26 $\mu\text{g/mL}$ for ascorbic acid. Since lower IC_{50} indicates stronger antioxidant activity, CuO nanoparticles showed better antioxidant potential than the algae extract, whereas ascorbic acid exhibited the highest activity. [49] A lower IC_{50} value indicates stronger DPPH radical scavenging ability, while a higher IC_{50} value indicates reduced scavenging potential.

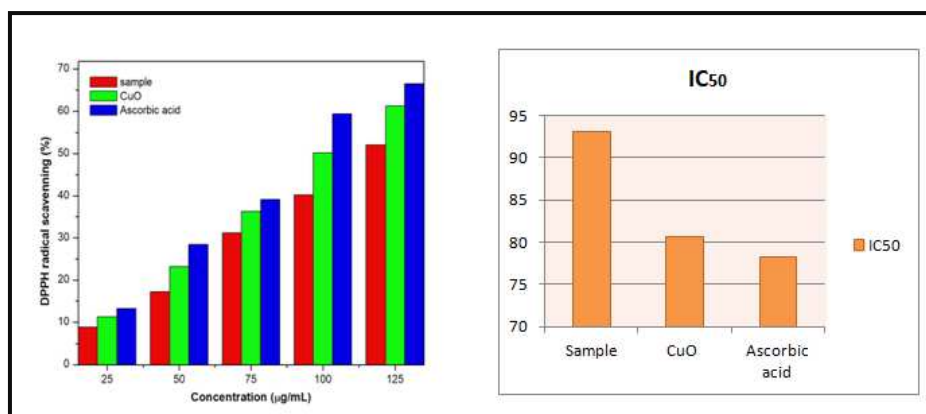


Fig.12. Antioxidant activity of DPPH assay synthesized CuO nanoparticles using red algae

5.2.2 H₂O₂ Radical scavenging Activity:

Fig. shows the H₂O₂ Radical scavenging activity showed a gradual increase with increase in concentration from 25 to 125 µg/mL, indicating concentration-dependent manner. At 25 µg/mL, the scavenging activity was found to be 9.06% for algae extract, 11% for CuO nanoparticles, and 12% for ascorbic acid. When the concentration was increased to 125 µg/mL, the inhibition significantly increased to 53.14 ± 0.11%, 61.06 ± 0.25%, and 63.24 ± 0.22%, respectively. The antioxidant efficiency was further evaluated using IC₅₀ values. The IC₅₀ values were found to be 90.26 µg/mL (algae extract), 80.32 µg/mL (CuO nanoparticles), and 71.26 µg/mL (ascorbic acid). Since lower IC₅₀ indicates stronger scavenging activity.

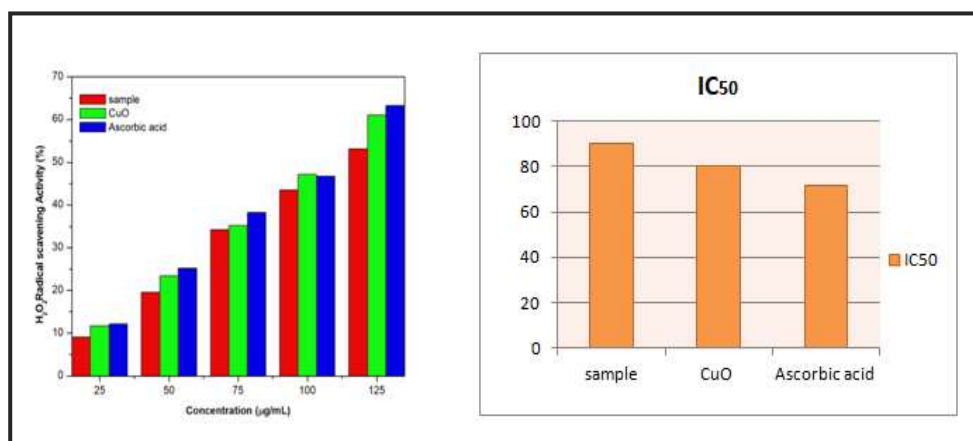


Fig.13. Antioxidant activity of H₂O₂ assay synthesized CuO nanoparticles using red algae

5.3 Antidiabetic Activity

The antidiabetic activity of copper oxide nanoparticles synthesized using algae extract *Gracilaria textorii* was assessed by α-amylase inhibition assay and evaluated at different concentrations. As shown in table 1 acarbose exhibited a concentration inhibition ranging from 26%, at 20 µg/ml to 90% at 100 µg/ml with an IG50 value of 44.84 µg/ml.

At 3750 µg/ml the greatest α -amylase inhibition was 81% whereas at 750 µg/ml the lowest inhibition was 17%. The standard acarbose has an IG50 value of 44.84 µg/ml, while the α -amylase inhibition IG50 value is 1986.2 µg/ml. The antidiabetic effect of the sulphated polysaccharides from seaweeds might be attribute to their inhibitory effects against α -amylase that retard the digestion of carbohydrate to delay the postprandial rise in blood glucose. [50] Seaweeds were reported to possess α -amylase a inhibitory activities. [51] The results obtained in the present study show that these seaweed species are a good source for antidiabetic agents.

Table: 1 Antidiabetic inhibitory of concentration of Acarbose and amylase activity inhibition:

Concentration of Acarbose (µg/mL)	Amylase activity inhibition (%)	Concentration of CuO I (µg/mL)	Amylase activity inhibition (%)
20	26	750	17
40	48	1500	41
60	66	2250	6
80	81	3000	76
100	90	3750	81
IG ₅₀	44.84	IG ₅₀	1986.2

In Fig.14 shows IG50 values were determined by plotting % inhibition against concentration and applying linear regression ($y=mx+c$). Among the algae extract, acarbose showed the strongest inhibitory activity with an IG50 value of 44.84 µg/ml and a high regression correlation ($R^2 = 0.9771$). *Gracilaria textorii* displayed moderate inhibition, with IG50 values of 1986.2 µg/ml ($R^2 = 0.9372$).

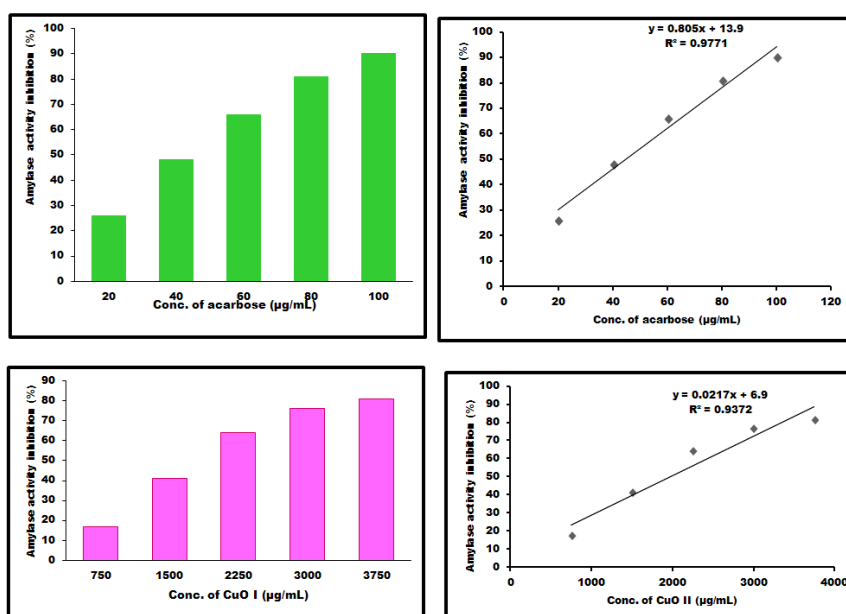


Fig. 14 Antidiabetic activity of IG50 values were determined by plotting % inhibition against concentration and applying linear regression ($y=mx+c$)

The results indicate that while algae-mediated copper oxide nanoparticles possess α -amylase inhibitory activity, their effectiveness is lower compared to the standard inhibitor acarbose. The observed differences between *Gracilaria textorii* suggest that variations in the algae extract composition, phytochemicals, or synthesis conditions may significantly influence nanoparticle properties, leading to differences in their biological activity.

Importantly, algae metabolites such as phenols, flavonoids, terpenoids, and polysaccharides play a dual role as reducing and stabilizing agents during nanoparticle synthesis. The α -amylase enzymes are essential to reduce hyperglycemia since their inhibition will affect the breakdown of carbohydrates to monosaccharides, to further simpler forms that disturb the blood sugar levels and give rise to high blood sugar.[52] The previous reports suggest that the marine algal compounds have the ability to inhibit the alpha amylase enzyme.[53] The resulting outcomes demonstrated the great potential of CuO as a powerful and efficient antidiabetic drugs towards the commercially available α -amylase enzyme and the biologically active enzyme.

6. Conclusion:

In this current study, convenient and affordable, eco-friendly method to synthesize copper oxide nanoparticles using two different algae *Gracilaria textorii* extracts through a green synthesis approach. The CuO nanoparticles were analyzed and characterized through UV–Vis spectroscopy, FT-IR, SEM coupled with EDAX, and XRD techniques. The UV–Vis spectrum revealed an SPR band at 272 nm attribute to CuO NPs. XRD confirms that the particle size of around 17.8 nm having monoclinic phase structure. SEM Morphology of irregular and agglomeration shape and EDAX confirm the peaks for copper (Cu) and oxygen (O) the formation of CuO Nanoparticles. The FTIR spectra of CuO NPs show the presence of bioactive functional groups that are present in the algae extracts. The biosynthesized nanoparticles demonstrated the CuO NPs exploited as photocatalyst exhibited excellent degradation efficiency methylene blue dye the percentage of dye algae found to be 76%. Cyclic voltammetric studies of the algae mediated CuO nanoparticles revealed clear oxidation and redox behavior, confirming their good electro activity nature. The antioxidant activity of DPPH H_2O_2 Radical scavenging activity showed a Strong antioxidant activity and antidiabetic study revealed that algae extract exhibited α -amylase inhibitory activity suggesting the potential as a phytomedicine to regulate blood sugar levels and prevent health complications in diabetic patients. The results obtained his biosynthesis technique can be a promising for the CuO nanoparticle and can be valuable in environmental, biotechnological, pharmaceutical and medical and further applications.

ACKNOWLEDGMENT:

I express my sincere gratitude to Dr.S.Sankaravadivu for their guidance and support throughout this research work I also thank and acknowledge the PG & Research Department of Chemistry, A.P.C. Mahalaxmi College for Women, Thoothukudi, for providing laboratory for synthesis nanoparticles and study for photocatalytic degradation, UV & FTIR Study V.O.Chidambaram college PG& Research Department of chemistry, Thoothukudi, Tamilnadu, XRD and Cyclic voltammetry study Avinashlingam Institute for the Home Science and Higher education for Women, Coimbatore, Tamilnadu, and Gandhigram Rural Institute, Dindigul, Tamilnadu, India, study for SEM & EDAX . Antioxidant activity study BIOMelTez Research & Development

Pvt Ltd, Kanyakumari. Antidiabetic activity study from Bioinnov Solutions LLP, Life Sciences Research and Development Centre, Salem, Tamilnadu.

Conflicts of Interest:

The authors declare no conflict of interest.

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