Biomimetic synthesis of copper nanoparticles using rhizome extract of *Corallocarbus epigaeus* and their bactericidal with photocatalytic activity

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Received: 21 November 2019 / Accepted: 22 April 2020 / Published online: 6 May 2020
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Abstract

The present study demonstrates the green synthesis of copper nanoparticles (CuNPs) from *Corallocarbus epigaeus* rhizome extract and its antioxidant and antibacterial activity against multi-drug resistant *Salmonella enterica* serovar Typhi. Bio-genic CuNPs were characterized by UV–Visible spectroscopy, transmission electron microscopy, Fourier transform-Infrared spectroscopy, X-ray diffraction, scanning electron microscopy with energy-dispersive X-ray spectroscopy. Obtained results confirmed spherical shaped CuNPs with an average diameter of 65–80 nm. Dose-dependent (2–10 mM) growth assay of *Salmonella enterica* serovar Typhi showed effective inhibition mechanism of CuNPs. The CuNPs exhibited tremendous degradation efficiency for the methylene blue (MB) almost 90% removal in 75 min illumination.

Keywords Antibacterial activity · Antioxidant activity · Photocatalytic activity · *Corallocarbus epigaeus* rhizome · CuNPs

1 Introduction

In recent times, nanosize-based materials and/or nanoma-terials were extensively studied because of their unique properties and applications in the fields of science and technology and medicine [1]. In particular, metal nano-particles have received significant attention due to their optical and electromagnetic properties [11]. Among the various metal nanoparticles, copper nanoparticles (CuNPs) have attracted tremendous attention due to their signifi-cant physicochemical, optical, catalytic and heat transfer properties [6, 14]. Moreover, CuNPs has been applied in various fields, including solar cells [16], energy storage [3], dye degradation [17], chemical manufacturing [37], and medicinal application [33].

The chemical mediated CuNPs synthesis involves the use of toxic chemicals which may pollute the environ-ment [26]. Thus, non-toxic, low cost and eco-friendly nature have received great attraction to the research-ers because of simple methodology, rapid synthesis, and improved stability compared to physicochemical methods. Several studies have been reported on the synthesis of CuNPs using the biological materials such as bacteria [10] fungi [4], edible mushroom [19] and plant extracts [5, 31]. Among the various biological resources, plant, and plant-derived materials are commonly used for CuNPs synthesis because of their ease of availability, cost-effective, compatibility and easy scale-up for com-mercial scale. Several plants and plant-derived materials such as *Azadirachta indica* [5], *Cissus quadrangularis* [21], *Quisqualis indica* [20], *Syzygium aromaticum* bud extract

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[24], and also many rhizomes like, *Rheum palmatum* L. rhizome extract [18], and ginger and garlic extracts El-Refai et al. [7] have been used for the synthesis of CuNPs. However, there are no reports on the synthesis of CuNPs using the rhizome extract of *Corallocarbus epigaeus*.

Corallocarbus epigaeus is a traditional medicinal plant practiced by ancient Siddha and Ayurvedic medicine for the treatment of snake bites, asthma, rheumatism, and also for the syphilitic disease. The shoot system of the plant is commonly used for medicinal practices, whereas rhizome is very effective compared to the shoot system. The rhizome is bitter in taste and contains many phytochemicals such as carbohydrate, phenols, steroids, sterols, alkaloid, saponin, tannins, and flavanoids [22] reported that *C. epigaeus* extract has both antioxidant and antimicrobial activity because the extract contains several phytochemicals and secondary metabolites. Since the *C. epigaeus* contains several phytochemicals and/or secondary metabolites, it could be used as biological reducing agents for CuNPs synthesis. However, the *C. epigaeus* rhizome extract mediated synthesis of CuNPs with effective antibacterial activity against MDR strain has not been reported previously. Hence, the objectives of the present study were (i) synthesis and characterization of CuNPs using *C. epigaeus* rhizome extract, and (ii) assess the antioxidant and antibacterial potential of CuNPs against multidrug-resistant (MDR) *Salmonella enterica* serovar Typhi (iii) evaluate the photocatalytic degradation of methylene blue (MB).

2 Materials and methods

2.1 Plant material and chemicals

The fresh rhizome of *C. epigaeus* was collected from Yercaud hills (11.8309° N, 78.2305° E), Tamil Nadu, India. The rhizome was thoroughly washed several times with tap water to remove the dust particles, shade dried for one week to remove the residual moisture, and ground to form a powder. The powder was stored in an airtight container at 4 °C and used for further studies. All other chemicals used in this study were of analytical grade and were purchased from Hi-Media Laboratories Pvt. Ltd (Mumbai, India).

2.2 *Corallocarbus epigaeus* rhizome extracts preparation

The rhizome powder (5 g) was dissolved in 100 mL of distilled water and boiled for 30 min at 50 °C. The extract was

filtered by Whatman No.1 filter paper, and the filtrate was used for CuNPs synthesis.

2.3 Synthesis of CuNPs

The CuNPs were synthesized according to [25] with minor modifications. Freshly prepared CuSO₄ solution (5 mM) (90 mL) was mixed with 10 mL of *C. epigaeus* rhizome extract in a 250 mL Erlenmeyer flask with constant stirring on a magnetic stirrer at 80–100 °C. Colour change of the reaction mixture was observed from deep blue to colourless and then to brick red and dark red on vigorous stirring for 12 h. The CuNPs were separated by centrifuging at 10,000 rpm and dried in the open air for 24 h. To optimize the experimental variables, CuNPs synthesis was conducted at various concentrations of rhizome extract (10–40 mL).

2.4 Characterization of CuNPs

Corallocarbus epigaeus rhizome extract mediated CuNPs were visually monitored and an absorbance spectrum was recorded using a UV–Visible spectrophotometer (Elico-164 PC) in the range of 200–500 nm. The size and morphology of synthesized CuNPs were observed by transmission electron microscope (TEM, FEI Tecnai TF 20 high resolution). The elemental composition of the particles was analyzed by scanning electron microscope-energy dispersive X-ray analysis (SEM-EDX- Jeol JSM 6390). Fourier transform-Infrared spectroscopy (FT-IR Shimadzu-8400) analysis was performed to understand the role of *C. epigaeus* biomolecules in the metal reduction. FT-IR spectrums were recorded with a range of 400–4000 cm⁻¹. X-ray diffraction (XRD) was recorded in the 2θ range of 30–80° using XRD6000, (Shimadzu) of Cu Ka radiation, the energy of which was 8.04 keV and wavelength was 1.54 Å.

2.5 Antioxidant assay

Antioxidant potential of rhizome extract and biosynthesized CuNPs was evaluated by 2, 2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) dot-blot method [29, 30]. The radical scavenging capacity towards DPPH was tested using thin-layer chromatographic (TLC) plates (Merck). Briefly, 10 µL of CuNPs sample was spotted onto TLC plates with the help of a microcapillary tube and allowed to dry for 3–5 min. The staining of silica gel TLC plates was carried out by placing them upside down for 10 s in 0.004% DPPH solution. The excess solution was removed with the blotting paper, and the plates were air-dried. The

formation of coloured zones on the TLC plate indicates the antioxidant potential of CuNPs.

2.6 Antibacterial assessment

The antibacterial activity of CuNPs was assessed according to Govarthan et al. [9] against multidrug-resistant (MDR) *Salmonella enterica* serovar Typhi (Accession No. KT037135). The MDR bacteria were inoculated in nutrient broth supplemented with different concentrations of CuNPs (2–10 mM), and the flasks were incubated at 37 °C for 12 h. The samples were collected at regular time intervals, and the bacterial growth rate was measured in terms of an increase in optical density (OD) at 600 nm. Control experiments were carried out in the absence of CuNPs.

2.7 Degradation of Methylene blue

The photocatalytic activity (PCA) of biosynthesized CuNPs was assayed by the degradation of methylene blue (MB) under sunlight irradiation as previously reported by Singh et al. [32] and Kayalvizhi et al. [13]. About 20 mg of biosynthesized CuNPs was added to 100 mL (100 mg/L) of MB while the control contained only for MB. The dye suspension was mixed thoroughly using a shaker for 1 h in dark condition. After, the subsequent mixture was allowed to remain underneath the sunlight and the intensity 850 W/m² between 10:00 and 11:15 am. At specified point intervals, 5 mL the dye sample was taken, and centrifuged at 10,000 rpm for 10 min, and the absorbance range (200–700 nm) was estimated using a UV–Visible spectrophotometer (Elico-164 PC). The degradation efficiency (%) has been determined by the following Equation.

$$\text{Degradation Efficiency (\%)} = \left(\frac{C_0 - C_t}{C_0} \right) \times 100$$

where, C_0 is the initial concentration and C_t are final MB concentrations with time t in the aqueous phase, respectively.

3 Results and discussion

The colour change of the reaction mixture from deep blue to brick red after 12 h of incubation primarily indicates the conversion of Cu into CuNPs. Figure 1 shows the UV spectra of biosynthesized CuNPs using a different concentration of *C. epigaeus* rhizome aqueous extract. The broad peak obtained at 280 nm further confirmed the formation of CuNPs. The results are consistent with the previous studies reported the surface plasmon resonance of CuNPs at

Fig. 1 UV–Vis spectra of biosynthesized CuNPs and rhizome extract

280 nm [23, 36]. TEM image (Fig. 2a) of the synthesized CuNPs shows the spherical, relatively uniform shape and size distribution of nanoparticles with a diameter range of 65–80 nm. This is very similar to those described in the previous report [25]. Figure 2b shows the SEM image of the particles is uniform distribution, nearly spherical in shape. The EDX showed in Fig. 2b revealed the strong signal in the copper region and confirmed the formation of CuNPs. CuNPs generally show a typical visual absorption peak around at 1 and 8 keV due to surface Plasmon resonance and also indicate that bio-synthesized CuNPs were free from impurity [5].

The *C. epigaeus* rhizome extract having bioactive compounds that might play a responsibility in the CuNPs synthesis can be confirmed by FT-IR spectrum [28]. Figure 3a represents the FT-IR spectra of *C. epigaeus* rhizome extract. The FT-IR analysis shows different characteristics peaks at 3441.16, 2923.0425, 2858.63, 2360.01, 1649.21, 1457.28, 1157.34, 1025.21, 778.28 cm⁻¹ between the range 400–4000 cm⁻¹. It was observed that peaks appeared at 3441.16 cm⁻¹ corresponded to the phenolic O–H group of rhizome extract. The peak appeared at 2923.04 cm⁻¹ was associated with C–H vibrations. The bands appearing at 1649.21 cm⁻¹ and 1457.28 cm⁻¹ are attributed to the formation of oxygen functional groups like highly conjugated (C=O) stretching in carboxylic groups. The peak at 1025.21 cm⁻¹ has corresponded to the presence of C–O stretching vibrations in the ester group present in the rhizome extract. The broad band 3441.16 cm⁻¹ is changed to 3433.44 cm⁻¹ (Fig. 3b). In addition; the prominent bands at 590.24 and 461.01 cm⁻¹ confirm the presence of the Cu vibration in the biosynthesized CuNPs [15, 27, 33].

Fig. 2 **a** TEM with histogram and **b** SEM-EDS images of CuNPs synthesized from rhizome extract

Fig. 3 FT-IR spectra of **a** rhizome extract and **b** CuNPs

Fig. 4 XRD pattern of CuNPs

XRD analysis was carried out to understand the nature of biosynthesized CuNPs, and the results are shown in Fig. 4. The XRD analysis shows a series of diffraction peaks at 32.77, 34.66, 38.72, 48.20, 52.24, 57.35 and 61.27 (2θ) corresponding to (110), (-111), (111), (-202) (020), (202) and (-113) planes, respectively, which are in good agreement with the diffraction data card JCPDS No. 05-0661. The average crystal size of 60 nm was calculated from Scherer equation. The XRD pattern of these peaks indicates the CuNPs is crystalline in nature, and one of the unassigned peaks were observed, it may be due to the fewer vibrating molecules in the rhizome extract. The XRD diffraction patterns showed in this study are in high-quality agreement with the previous research reported for the biosynthesis of CuNPs [2, 26].

Antioxidant activity of rhizome and CuNPs were estimated semi-quantitatively by a rapid DPPH staining method using TLC. Samples were applied as a dot on a TLC layer that was then stained with DPPH solution (Fig. 5a). The appearance of white colour spot vs. a purple background has a potential value for the indirect evaluation of the antioxidant capability of rhizome and CuNPs in the dot blots. Biosynthesized CuNPs showed high antioxidant potential (2.5 cm) comparing with the rhizome extract (1.5 cm) [29]. The bactericidal activity of synthesized CuNPs was investigated against MDR *S. Typhi* human bacterial pathogen which shown in Fig. 5b. In this study, the CuNPs at 5 doses (1–5 mM) along with rhizome extract and was examined. The bactericidal result indicates that the growth inhibition noticed was minimum inhibition found in 2 mM and maximum observed in 10 mM of CuNPs. The strong bactericidal activity of CuNPs against MDR *Salmonella enterica serovar Typhi* noticed that the increasing concentrations of CuNPs effectively encountered the bacterial population. The results are consistent with studies reporting the antibacterial activity of CuNPs [8, 36]. Tover Corona et al. [34] reported that the antibacterial activity of CuNPs depends both on the surface on released ions and/or accumulated in the cell wall membrane caused permeability, resulting in bacteria lysis, through the release of lipopolysaccharide and membrane protein channels. Kaviya et al. [12] reported that the CuNPs may collapse the structure of the cell membrane of the bacteria resulting in bacteria lysis.

The photocatalytic activity (PCA) of the biosynthesized CuNPs was evaluated using methylene blue (MB). The UV spectrum of MB has an absorption maximum at 664 and 294 nm. Figure 5c showed the degradation efficiency of the MB on the rhizome extract mediated synthesized CuNPs as photocatalysts as a behavior of reaction time. The percentages of dye degradation by CuNPs under the source of sunlight have obtained about 90% after 75 min.

Fig. 5 **a** Antioxidant activity of rhizome extract and CuNPs, **b** Anti-bacterial activity of CuNPs against MDR bacteria **c** spectrum of photodegradation of methylene blue

Earlier reports on photocatalytic investigate revealed that the PCA of the CuNPs would depend on the crystalline structure, morphology, and size of the particles [35].

4 Conclusions

In summary, CuNPs were synthesized successfully using a simple, eco-friendly, rapid green methods using rhizome extract of *C. epigaeus*. The biogenic CuNPs were spherical in shape and crystalline nature with effective antibacterial activity against multidrug-resistant *S. typhi*. The green engineered CuNPs showed potential bactericidal activity against the human pathogen. The green synthesized CuNPs showed very good photocatalytic efficiency against methylene blue dye. It is suggested that the CuNPs obtained from the rhizome extract of *C. epigaeus* could be used as an effective antibacterial agent to control and/or kill the population of multi-drug resistant bacteria.

Acknowledgements This work is partially supported by the Department of Biotechnology, Mahendra Arts and Science College (Autonomous), and the Department of Science and Technology, Government of India (DST FIST sponsored-Ref. No. SR/FST/College-232/2014).

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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