ORIGINAL RESEARCH PAPER

Microbiology

BIOSYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL POTENTIAL STUDIES OF COPPER OXIDE NANOPARTICLES PRODUCED FROM *CHLORELLA VULGARIS*

KEY WORDS: Copper Oxide; *Chlorella*; Nanoparticles

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ABSTRACT

The applications of nanoparticles are gaining a significant role in the current scenario as they show excellent anti-bacterial and antibacterial and antitumor activities. Biosynthetic methods of nanoparticles have emerged as a simple alternative to more complex chemical synthetic procedures to obtain nanomaterials. In the present scenario, algae are being used largely for the nanoparticles synthesis. In the present study, copper oxide nanoparticles (Cuo NPs) are biosynthesized by using *Chlorella vulgaris*, a marine alga. Further, the characterization, optimization and antimicrobial studies of the nanoparticles were carried out. The biosynthesized Cuo NPs were confirmed visually by the appearance of dark brown colour formation in the mixture added with copper acetate. The existence of nanoparticles was confirmed by UV visible spectroscopy at 540 nm. Biosynthesized nanoparticles were characterized by SEM, EDAX and XRD. Further, antimicrobial potential studies of synthesized Cuo NPs were carried out against selected bacteria and fungi.

INTRODUCTION

In recent years, nanoparticles have achieved a huge attention and influenced material science considerably. It seems, this dominance will continue in the future years because of fundamental and technological importance with implem enting incessant researches in this field. Nanotechnology is the branch of science and technology that deals with the production of substances in size less than 100 nm scale as nanoparticles (Mohanpuria *et al*., 2008).

Among various transition metal oxides, copper oxide has attracted greater attention due to its antimicrobial activity (Azam *et al*., 2012), anticancer activity (Sankar et al., 2014). Various physical and chemical process have been extensively used to produce Copper oxide Nanoparticles (CuO NPs), however the use of toxic chemicals on the surface of NPs and non-polar solvents in the synthesis procedure limits their applications in medical fields. Therefore, the interest in this field has shifted toward use of algae to produce biocompatible, nontoxic and eco-friendly CuO NPs. The use of algae in the synthesis of NPs has encouraged the designing of simple, green, cost and time effective approaches thereby, minimizing the use of chemicals and solvents. The biomedical application of biosynthesized NPs using algae is considerably becoming more significant owing to their antibacterial, antifungal, anticancer, and wound healing activity.

Algae are able to absorb the metal and it is the reason, it is used for the synthesis of nanoparticles. Algae contains large amount of reducing agent which is able to reduce metal salts to their respective metal nanoparticles (Sangeetha *et al*., 2014; Bilal *et al*., 2018). Various phytochemicals in the algae play an important role in the synthesis of nanoparticles (Patel *et al*., 2015).

Hence, in the present study attempts were made to utilize the potential of cell-free extracts of *Chlorella vulgaris* as a bio-factory for the CuO NPs synthesis. The biosynthesized CuO NPs were characterized by UV-visible spectroscopy, Scanning electron microscopy, X-ray diffraction, and Energy Dispersive X-ray Analysis. Furthermore, antimicrobial activity of CuO NPs has examined by Minimum Inhibitory Concentration (MIC) against bacteria and fungi.

MATERIALS AND METHODS

MICROORGANISM AND CULTURE CONDITIONS

Chlorella vulgaris culture was obtained from Madras Christian College, Chennai in a sterile conical flask and cultured in Bold Basal medium. The growth of alga was maintained at 28°C with 4000 lux light intensity for 30 days. After 30 days of growth biomass was harvested, it was dried in hot air oven at 45°C for 24 h and homogenized. The fine powder was stored at 18°C for further studies (Fig. 1).

PREPARATION OF ALGAL EXTRACT

Algal extract was prepared by adding 1 g of *Chlorella* powder to 100 ml of DMSO and kept in magnetic stirring for 1 hour. Then the solution was filtered using Whatmann filter paper and the extract was collected (Fig. 2).

SYNTHESIS OF COPPER OXIDE NANOPARTICLES

Around 100 ml of 1m M copper acetate was taken and 2 ml of *Chlorella vulgaris* was added drop-wise. The reaction mixture were continuously stirred and incubated for 42 hours at 80-100°C. Change of colour from light blue to brown indicates the formation of Copper oxide nanoparticles. The reaction mixture was centrifuged at 14,000 rpm for 20 min. Supernatant was discarded and the pellet was washed with water and kept for centrifugation. This step was repeated three times. Copper oxide nanoparticle was settled at the bottom of the tube. The pellet was then dissolved in DMSO and UV-Visible spectrum was taken.

CHARACTERIZATION OF CUO NPS

Synthesized copper oxide nanoparticles, were examined under UV-Visible spectrum. The nanoparticles were scanned in the wavelength ranging from 200 to 600 nm. The shape and size of the synthesized CuO NPs were determined by SEM analysis. XRD analysis was carried out to characterize the crystalline nature of CuO NPs. EDAX analysis were done to determine the elemental analysis of the prepared nanoparticles.

ANTIMICROBIAL STUDIES OF CUO NPS

Antimicrobial activity of synthesized CuO NPs was evaluated by minimum inhibitory concentration method against two gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) as well as with two fungal cultures (*Candida albicans* and *Aspergillus niger*).

RESULTS AND DISCUSSION

In this study, *Chlorella vulgaris* was used for biogenic synth esis of CuO NPs and were examined for antimicrobial activity against bacteria and fungi. Algae have been used extensively for the synthesis of nanoparticles.

SYNTHESIS OF CUO NPS

The synthesised nanoparticles were confirmed by observing the characteristic colour change. Initially the solution was blue in colour, after a period of 8 hours blue colour disappeared, and finally after incubation up to 24 hours, light brown colour appeared which confirmed the presence of formation of copper oxide nanoparticles.

Copper acetate is used as a precursor molecule for the synthesis of CuO NPs. Similar work have been carried out using copper acetate as a precursor molecule to produce copper oxide nanoparticles by aqueous precipitation method (Amrut *et al.*, 2010; Saran *et al.*, 2017).

CHARACTERIZATION STUDIES OF CUO NPS

UV-visible spectrum of copper oxide nanoparticles exhibited absorption peak around 540 nm. Rahman, *et al.* (2009) and Swadzba-Kwasny, *et al.* (2012) have reported similar pattern of peak for the confirmation of copper oxide nanoparticles in their studies.

X-Ray Diffraction study was done to identify the crystalline nature of the nanoparticle. Using Scherrer formula, the size of the synthesized CuO nanoparticles were calculated and the average size was found to be 19.98 nm. XRD pattern of synthesised CuO nanoparticles is shown in figure 3. In the present work, X-ray diffraction patterns were observed to be at 2θ = 32, 35, 38, 49, 53, 58 and 62 which are in accordance with the monoclinic crystal structure of copper oxide (Padil and Černík, 2013). The present experimental results were found to be in agreement with the reported diffraction patterns of CuO NPs prepared by Das *et al.* (2013).

The morphology of the nanoparticles was determined by using SEM. The SEM image of the synthesized CuO NPs was found to be uniformly circular. The average particle size was calculated using imageJ and it was found to be 40.0 ± 0 nm. The SEM image of the CuO NPs is illustrated in figure 4.

EDAX analysis were done in order to determine the elemental analysis of the prepared nanoparticles, and it was confirmed that the prepared nanoparticle contains copper and oxide as the major component as per the requirement along with trace amount of carbon as minor impurity (most common contaminant). EDAX analysis is depicted in figure 5.

ANTIMICROBIAL STUDIES OF CUO NPS

In the current study, Minimum Inhibitory concentration of CuO NPs for gram-negative and gram-positive bacteria was found to be at a concentration of 250µg/ml and for fungi at a concentration above 500µg/ml was recorded. The MIC values of copper oxide nanoparticles are shown in table 1.

Antimicrobial properties have been proved in different nanoparticles such as silver, zinc, gold, and copper from different biological sources. There is no report evidenced for the synthesis of copper oxide nanoparticles from *Chlorella vulgaris*. Hence, in this study *Chlorella vulgaris* has been selected as a source for the synthesis of CuO Nps.

In the current research, Gram-negative bacteria (*E.coli* and *K.pneumoniae*), that causes UTI and Gram-positive bacteria (*S.aureus*), which cause wound infections and *C.albicans* which has clinical importance have been selected to find the antimicrobial property of Cuo NPs. In many reports, the above mentioned organisms were used to study the antimicrobial efficiency (Jayandran *et al.*, 2015).

Maqusood Ahamed *et al.* (2014) reported the lowest MIC (31.25 µg/ml) was for *E. coli* and *E. faecalis* while the highest MIC (250 µg/ml) was for *K. pneumoniae* in their work. The MIC values of CuO NPs found in this study were slightly higher than CuO NPs produced from gum karaya (Vellora *et al.*, 2013).

Since the biosynthesized copper oxide nanoparticles from *Chlorella vulgaris* exhibited antimicrobial activity, further work can be carried out such as anti-oxidant, anti-cancer activity and wound healing efficiency. Moreover, biosynthesis of copper oxide nanoparticles from *Chlorella vulgaris* is cost effective and eco-friendly compared with chemical synthesis method.

CONCLUSION

In the present study, CuO nanoparticles with monoclinic structure were synthesized by using *Chlorella vulgaris* and the formation of CuO NPs was confirmed by UV-Visible spectroscopy. XRD analysis of the CuO NPs demonstrated the crystalline nature and particle size determined as 19.98 nm. The surface morphology of the synthesized CuO nanoparticles was found to be uniformly circular by SEM analysis. EDAX analysis of CuO NPs demonstrated the elemental composition and also shown trace amount of carbon as minor impurity (most common contaminant). MIC analysis indicates the antimicrobial activity of CuO NPs for combating bacteria and fungi.

Fig 1: Dried Powder of *Chlorella vulgaris*

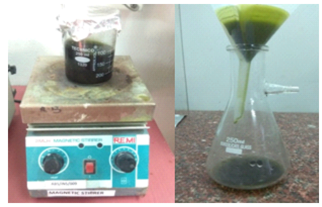

Fig 2: Preparation of Algal Extract

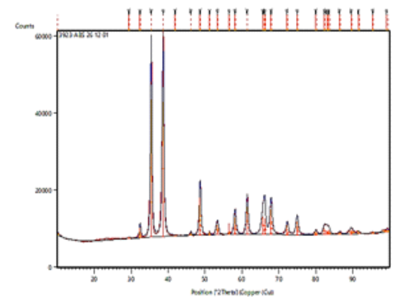

Fig 3: XRD Analysis of CuO NPs

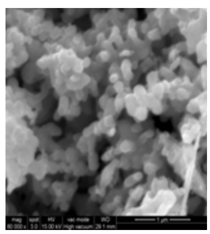

Fig 4: SEM Analysis of CuO NPs

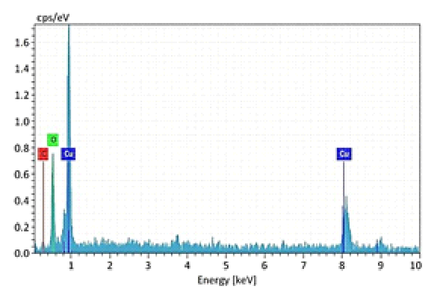

Table 1: MIC values of CuO NPs against gram negative, gram positive bacteria and fungi

S.No	Organism	Concentration of CuO NPs (µg)					
		15.625	31.25	62.5	125	250	500
1	*E.coli*	++	++	+	+	-	-
2	*K.pneumoniae*	++	++	++	++	-	-
3	*S.aureus*	+++	+++	+++	+++	-	-
4	*B.subtilis*	++	++	+	+	-	-
5	*C.albicans*	+++	+++	+++	+++	+++	+++
6	*A.niger*	+++	+++	+++	+++	+++	+++

Test 5497

Element	At. No.	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
Cu	29	48.29	75.97	42.99	2.77	5.74
O	8	12.72	20.02	44.99	3.42	26.88
C	6	2.55	4.02	12.02	1.94	76.14
		63.57	100.00	100.00		

Fig 5: EDAX Analysis of CuO NPs

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