ORIGINAl RESEARCH PAPER

BIOSYNTHESIS, CHARACTERIZATION AND ANTImICROBIAL POTENTIAL STUDIES OF COPPER OXIDE NANOPIRACLES PRODUCED FROM CHLORELLA VULGARIS

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ABSTRACT

The applications of nanoparticles are gaining a significant role in the current scenario as they show excellent 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) were 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 implementing 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 (Mohananpuria 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 processes 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 alga 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 biofactory 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 NANOPIRACLES

Around 100 ml of 1m M copper acetate was taken and 2 ml of Chlorella vulgaris was added drop-wise. The reaction mixture was 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 examines 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
(31.25 µg/ml) was found to be the MIC 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 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 antimicrobial activity of CuO NPs for combating bacteria and fungi.
Table 1: MIC values of CuO NPs against gram negative, gram positive bacteria and fungi

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism</th>
<th>Concentration of CuO NPs (μg)</th>
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<tbody>
<tr>
<td>1</td>
<td>E.coli</td>
<td>++ ++ + + - -</td>
</tr>
<tr>
<td>2</td>
<td>K.pneumoae</td>
<td>++ ++ ++ ++ -</td>
</tr>
<tr>
<td>3</td>
<td>S.aureus</td>
<td>+++ +++ +++ ++ -</td>
</tr>
<tr>
<td>4</td>
<td>B.subtilis</td>
<td>++ ++ + + - -</td>
</tr>
<tr>
<td>5</td>
<td>C.albicans</td>
<td>+++ +++ +++ +++ +++ +++</td>
</tr>
<tr>
<td>6</td>
<td>A.niger</td>
<td>+++ +++ +++ +++ +++ +++ +</td>
</tr>
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</table>

Test 5497

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<tr>
<th>Element</th>
<th>At. No.</th>
<th>Mass %</th>
<th>Mass Norm.</th>
<th>Atom %</th>
<th>Abs. error (%)</th>
<th>Rel. error (%)</th>
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<tbody>
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<td>Cu</td>
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<td>42.99</td>
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<td>O</td>
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<td>20.02</td>
<td>44.99</td>
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<tr>
<td>C</td>
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<td>15.02</td>
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<td>76.14</td>
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63.57 100.00 100.00

Fig 5: EDAX Analysis of CuO NPs

REFERENCES