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Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity

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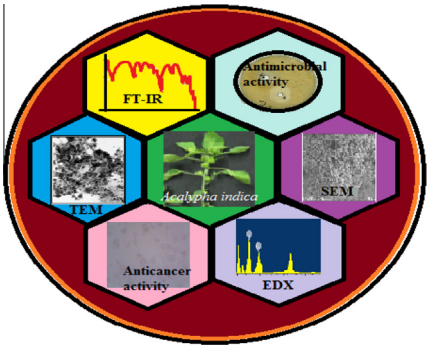
HIGHLIGHTS

- Highly stable copper oxide nanoparticles are successfully synthesized.
- *A. indica* mediated copper oxide nanoparticles showed antimicrobial activity.
- *A. indica* mediated copper oxide nanoparticles have anticancer activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Copper oxide nanoparticles were synthesized by biological method using aqueous extract of *Acalypha indica* leaf and characterized by UV–visible spectroscopy, XRD, FT-IR, SEM TEM and EDX analysis. The synthesised particles were highly stable, spherical and particle size was in the range of 26–30 nm. The antimicrobial activity of *A. indica* mediated copper oxide nanoparticles was tested against selected pathogens. Copper oxide nanoparticles showed efficient antibacterial and antifungal effect against *Escherichia coli, Pseudomonas fluorescens* and *Candida albicans*. The cytotoxicity activity of *A. indica* mediated copper nanoparticles was evaluated by MTT assay against MCF-7 breast cancer cell lines and confirmed that copper oxide nanoparticles have cytotoxicity activity.

Introduction

Green synthesis of nanoparticles is budding into an important approach in nanotechnology [1,2]. Green chemistry method highlights the usage of natural organisms as reliable, simple, nontoxic and eco-friendly [3,4]. Hence, investigators have focused the synthesis of nanoparticles using biological systems in the last years

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[5]. Synthesis of nanoparticles by using microorganisms, enzyme and plant or plant extract, have been proposed by several authors [6,7]. In the biological methods, it is found that the extracts of living organisms act both as reducing and capping agents in the synthesizing process of the nanoparticles [8]. Copper oxide nanoparticles are used as gas sensors, catalysis, batteries, high temperature superconductors, solar energy conversion tools, etc. [9–11]. It is very stable, strong and has a longer shelf life compared to organic antimicrobial agents [9,12]. Cioffi et al. [13] has reported the antifungal and bacteriostatic properties of copper nanoparticles/polymer composites. Berntsen et al. [14] studied the impaired cell viability in human airway smooth muscle cells and observed that reduced in cell contractility occurred due to exposure of copper oxide nanoparticles. *Acalypha indica* L. (family: *Euphorbiaceae*) is commonly distributed throughout the plains of India. It has been reported to be beneficial in treating pneumoniae, asthma, rheumatism and several other ailments [15].

In this research paper we have reported biosynthesis of copper oxide nanoparticles using aqueous extract of *A. indica* leaf. The present study is the continuation to assess the antimicrobial and cytotoxicity activities of *A. indica* mediated copper oxide nanoparticles.

Materials and methods

Materials

All the chemical reagents (analytical grade) were purchased from sigma–aldrich chemicals, India. The bacterial and fungal strains were obtained from Department of Microbiology, Karpagam University, Coimbatore, Tamil Nadu. Bacterial and fungal cultures namely *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris, Pseudomonas fluorescens* and *Candida albicans* were maintained in the respective medium.

Synthesis of copper oxide nanoparticles

A. indica plants were collected from in and around Karpagam University, Coimbatore, Tamil Nadu, India. 100 g of leaves were washed with tap water, ground and boiled with 500 ml of de-ionized water for 10 min. Finally the product was filtered and stored in freezer for further investigations. A 50% of leaf extract was made up to 250 ml. The analytical grade copper sulphate solution was prepared using de-ionized water and was mixed in the 50% leaf extract under constant stirring using magnetic stirrer. The mixture of this solution was kept under vigorous stirring at 100 °C for 7–8 h. After this process, a brownish black colour product obtained. The solid product obtained was washed twice with de-ionized water and dried at 80 °C for 8 h. Finally the dried powder was stored in properly labelled containers and used for further studies.

Characterization of copper oxide nanoparticles

UV–vis spectrophotometer (UV-2450, Shimadzu) was used to determine the optical property of copper oxide nanoparticles in 200–800 nm wave length range. The X-ray diffractometry (XRD) study was done using Perkin–Elmer spectrum one instrument. The functional groups in the copper oxide nanoparticles were predicated by Fourier transform infrared (FT-IR) spectrometer (Perkin–Elmer 1725×). Scanning electron microscopy (SEM) (Model JSM 6390LV, JOEL, USA) and Transmission electron microscope (TEM) (JEOL JEM-3100F) were used to study the morphology and size of the copper oxide nanoparticles. Energy dispersive X-ray spectrometer (RONTEC's EDX system, Model QuanTax 200, Germany) was used for elemental analysis of copper oxide nanoparticles.

Determination of antimicrobial activity of copper oxide nanoparticles

The antimicrobial activity was determined by well diffusion method [16]. The Muller Hinton Agar plates were prepared and 100 µl of microbial culture was swabbed. After 5 min the well (5 mm size) was cut by gel puncher and a volume of 100 µl (25 µg/ml) of the copper oxide nanoparticles was added into the well. The effects were compared with that of the standard antibiotic tetracycline (positive control) at a concentration of 10 µg/ml. The plates were incubated at 37 °C for 24 h (bacteria) and room temperature for 48 h (fungi). The assessment of the antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the well and the mean values are recorded.

Determination of cytotoxicity studies of copper oxide nanoparticles

Human breast MCF-7 cell lines (cell culture) were obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in Eagles Minimum Essential Medium (EMEM) added with FBS (10%, v/v) at 37 °C in a CO_2 incubator (95% air, 5% CO_2 and 100% relative humidity). In order to evaluate the cytotoxic effect of the green synthesised copper oxide nanoparticles against MCF-7 cells, the cells were collected in the exponential stage of growth, seeded into 96-well plates (15,000 per well) and permitted to adhere for 48 h. Then, Different concentrations (6.5, 12.5, 25, 50, 100 µg/ml) of *A. indica* mediated copper oxide nanoparticles were added to the desired wells and incubated for 48 h. A 20 µl of EMEM medium having MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Later, the medium was altered with 100 µL of DMSO, and optical densities were measured at 570 nm. All studies were performed in triplicates and expressed as the mean ± standard error.

Results and discussion

Synthesis and characterization of copper oxide nanoparticles

Fig. 1 shows the UV–Visible absorption spectrum of green synthesized copper oxide nanoparticles. It has an optical absorbance range around 220 nm. The XRD analysis of green synthesized copper oxide nanoparticles is shown in Fig. 2. The XRD peaks were obtained at (110), (111), (200), (202), (020), (202), (113), (311), (220) and (400) Bragg's reflection based on the crystal of copper

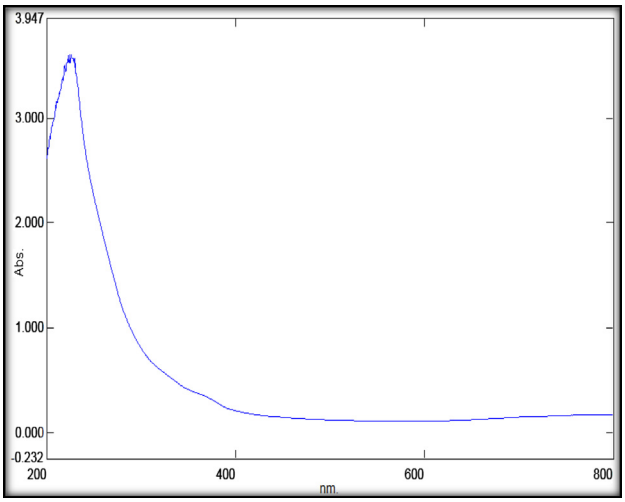

Fig. 1. UV spectra of *Acalypha indica* mediated copper oxide nanoparticles.