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Research Article

Synthesis, characterization and anticancer activity of copper nanobiocomposite synthesized by leaf extract of *Catharanthus roseus*

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Abstract

Nanotechnology has elevated the standards of treatment for various diseases especially cancer. The phytoconstituents of *Catharanthus roseus* can be used as antimicrobial, antioxidant, antifeedent, antisterilty and anticancer agents. It has high alkaloid content where more than 70 types where monoterpene indole alklaloids, vinblastine and vincristine can be used for the treatment of leukemia in children, breast and lung cancer. Copper oxide nanoparticles have gained received greater attention due to their promising properties. In the present work, copper nanocomposite was biosynthesized by the aqueous leaf extract of *Catharanthus roseus.* The biologically reduced copper bound alkaloid (phytoconstituent) nanocomposite was confirmed by UV spectroscopy, the functional group present in the nanocomposite was analyzed using Fourier Transform Infrared Spectroscopy, crystalline structure of the nanocomposite was characterized using X-ray diffraction crystallography. The anticancer activity against MCF-7 cell line was studied.

Keywords: Copper nanocomposite; Biosynthesis; *Catharanthus roseus;* Characterization; Anticancer activity.

Introduction

Nanoparticles have distinct properties offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology. Nanotechnology has been utilized in medicine for therapeutic drug delivery and the development of treatments for a variety of diseases and disorders. Nanoparticles attract scientists across many disciplines due the opportunity to engineer the properties and make it is compatible for various applications. Attachment of biologically active molecules, targeting sequences, fluorescents and biocompatible coatings on nanoparticles makes it as a versatile nanocarrier [1-3].

Copper nanoparticle synthesis specifically has attracted more interest compared to other nanoparticle synthesis because of their useful properties achievable at much less cost than silver and gold [4-5]. Copper nanoparticles acts as an anti-biotic, anti-microbial, and anti-fungal agent when added to plastics, coatings, and textiles [6-8].

Catharanthus roseus (L.) is a terpenoid indole alkaloid producing plant belongs to the Apocynaceae Family. *C. roseus* has been used to treat a wide assortment of diseases. Research shows that *C. roseus* contains over 130 compounds, and many of which have cytotoxicity. *C. roseus* has become a model species for the study of secondary metabolism in plants. The interest in this species arises from its therapeutic role as the source of the anticancerous alkaloids vinblastine and vincristine. The anticancer activity of these alkaloids is attributed to their ability to disrupt microtubules, causing the dissolution of mitotic spindles and metaphase arrest in dividing cells [9-12]. Thus the present work was focused on synthesis of copper nanobiocomposite by leaf extract of *C. roseus* and characterization of its anticancer activity against MCF cell line.

Materials and methods

Synthesis of copper nanobiocomposite using leaf extract of C. roseus

The *C. roseus* leaf was collected and ground with mortar and pestle using distilled water. The gound mixture was filtered and the filtrate was collected. For the preparation of copper nanoparticle, 50 ml of copper sulphate and 50 ml of leaf extract were mixed together and heated to 80°C for 45 min. It was further observed for a color change from green to greenish brown for the confirmation of the CuO nanoparticle

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formation [13]. The CuO was collected by centrifugation of mixture at 8000 rpm for 15 min. The sample was further lyophilized.

Characterization of copper nanobiocomposite

Various properties of the copper nanobiocomposites were observed by the following characterization. The surface Plasmon's of the nanocomposites was investigated by SYSTRONICS Double beam UV-visible spectrophotometer 2201 obtaining spectrum from 300 to 800 nm. The Fourier Transform Infrared Spectroscopic (FT-IR) analysis was used to characterize the functional groups of the nanocomposite using BRUKER α-T FT-IR spectrophotometer. The lyophilised samples were mixed with KBr to form discs under high hydraulic pressure. These discs were scanned for 400 to 4000 cm-1 to obtain FT-IR spectra. The crystalline nature of the copper nanobiocomposite was studied using X-Ray Diffraction (XRD) analysis.

Anticancer activity of copper nanobiocomposite

The anticancer activity of the synthesized copper nanobiocomposites was studied against MCF-7 cancer cell line using MTT assay. The MTT assay is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product [14]. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum at 37ºC in humidified atmosphere with 5% CO_2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2×10^4 cells/well and allowed to attach overnight at 37ºC. The medium was then discarded and cells were incubated with different concentrations of copper nanobiocomposite obtained using using leaf extract of *C. roseus* (15, 100, 150 µg) for 24 hrs. After the incubation, medium was discarded and 100 µl fresh medium was added with 10 µl of MTT (5 mg/ml). After 4 hours, the medium was discarded and 100 µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570 nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Results and discussion

The reduction of the metal precursor copper sulphate by the leaf extracts of *Catharanthus roseus* copper nanoparticles was confirmed by change in colour of the colloidal solution from green to greenish brown.

Characterization of copper nanobiocomposite using UV spectrophotometer

The copper nanobiocomposite colloid is in the range of 200 to 800 nm showing that the absorption was observed in the visible light region (Fig. 1). The peak obtained at 363.2 proves the existence of copper nanobiocomposites.

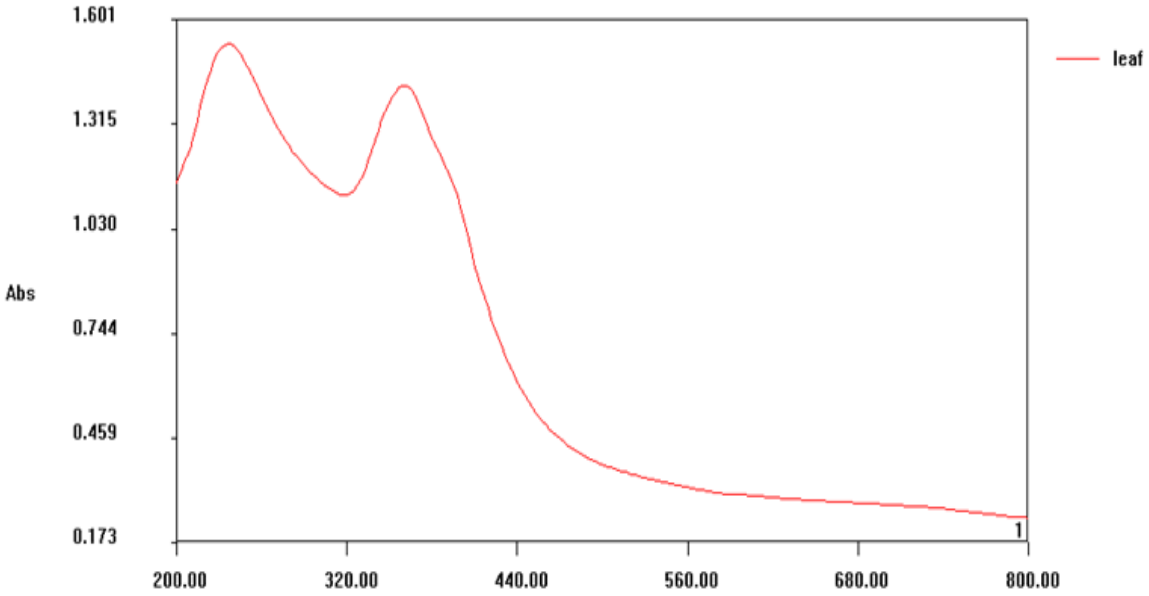

Figure 1. UV spectrum of copper nanbiocomposites synthesized using leaf extract of *C. roseus*

Characterization of copper nanobiocomposite using FT-IR analysis

The FT-IR spectra of copper nanobiocomposite obtained using leaf extracts of *C. roseus* displayed 10 peaks (Figure 2). The peak 3370 cm^{-1} showed the presence of $–NH_2$ in aromatic amines, primary amines and amides. The peak 2936 showed the presence of $–CH_3$ and $–CH_2$ in aliphatic compounds. The peak showed 1653 cm^{-1} showed C=C in alkenes. The peak 1541 cm^{-1} displayed very strong –NH in secondary amines and triazine compounds. The peak 1451 cm^{-1} showed benzene ring in aromatic compounds. The peak 1244 showed the presence of epoxides. The peak 1162 cm^{-1} displayed the presence of C-O-C in ester, lactones. The 1058 cm^{-1} showed the presence of C_2-O-H in primary alcohol. The peak 604 showed the Ar-OH in phenols.

XRD analysis of copper nanobiocomposite using FT-IR analysis

The XRD analysis was used to determine metallic nature and monoclinic structure of the synthesized nanoparticles. The XRD analysis of the copper nanobiocomposite synthesized using leaf extracts of *C. roseus* showed peaks 10.32, 11.08, 14.08, 24.84, 31.48, 33.52. These peaks confirm the nano-crystalline nature of the synthesized copper nanobiocomposite (JCPDS File No: 048-1548).

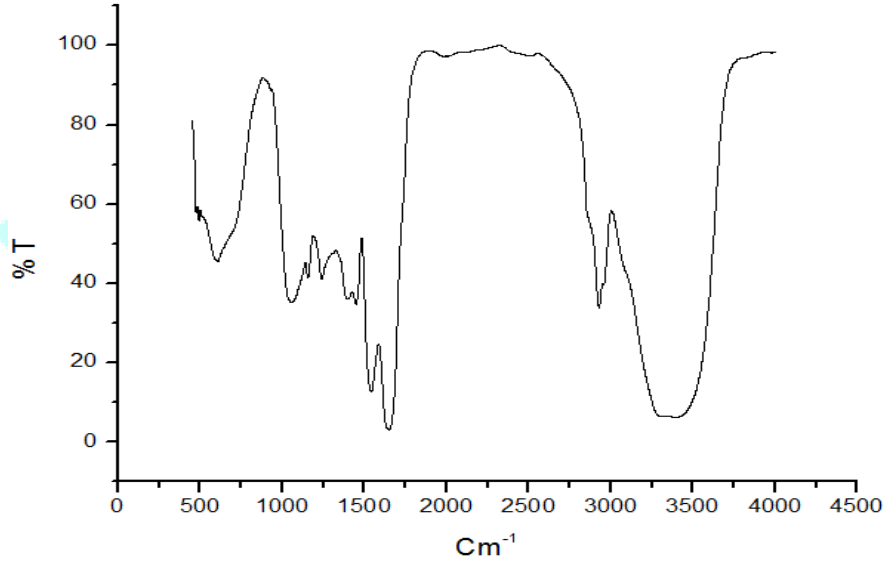

Figure 2. FT-IR analysis of copper nanbiocomposites synthesized using leaf extract of *C. roseus*

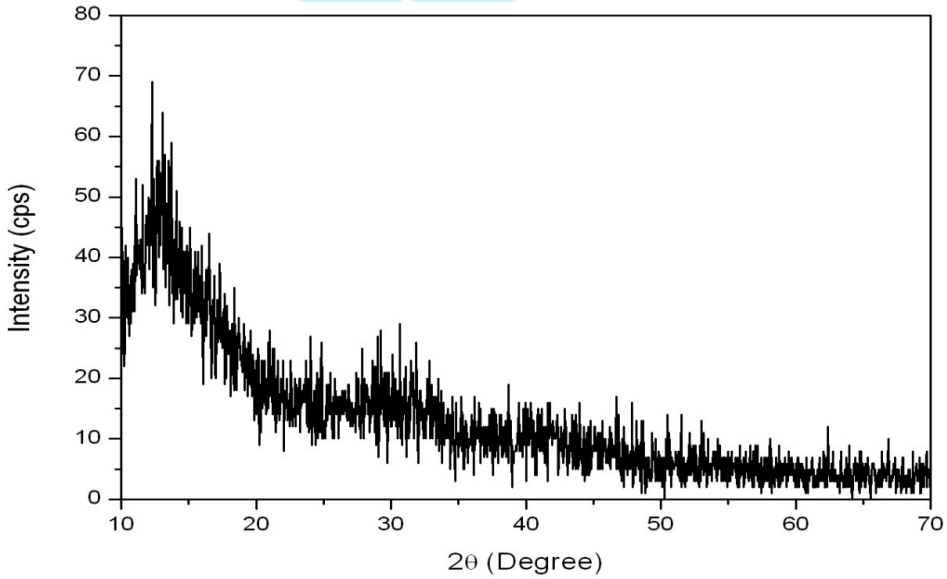

Figure 3. XRD analysis of copper nanbiocomposites synthesized using leaf extract of *C. roseus*

Anticancer activity of copper nanobiocomposite of leaf extract of C. roseus against MCF-7 cancer cell line

The anticancer activity of copper nanobiocomposite synthesized using leaf extract of *C. roseus* was studied against MCF-7 cancer cell line using MTT assay. The MTT assay results obtained for control (Figure 4), positive control (Figure 5) and copper nanobiocomposite of leaf extract (Figure 6) showed 27.91, 49.23, and 68.76 % for 50, 100 and 150 µg (Figure 7). This shows that as the concentration of the sample increased the cell toxicity also had increased, thus proving that there is a direct proportionality between the concentration of the sample and cytotoxicity. The positive control used was cyclophosphamide which displayed 73.82 % cytotoxicity.

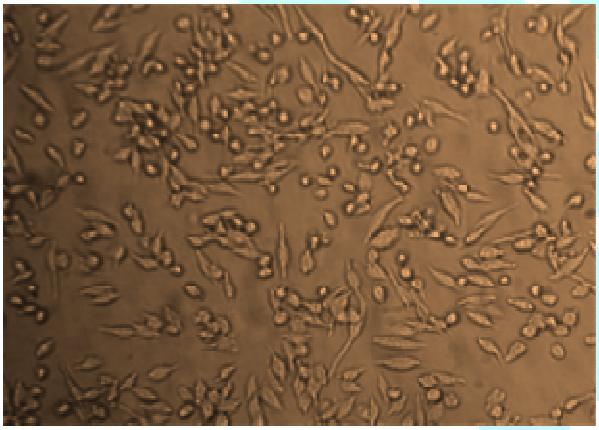

Figure 4. MTT assay on control of MCF-7 cancer cell line

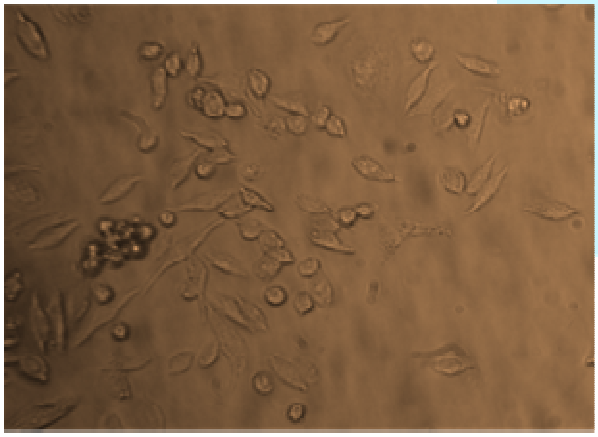

Figure 5. MTT assay of positive control on MCF-7 cancer cell line

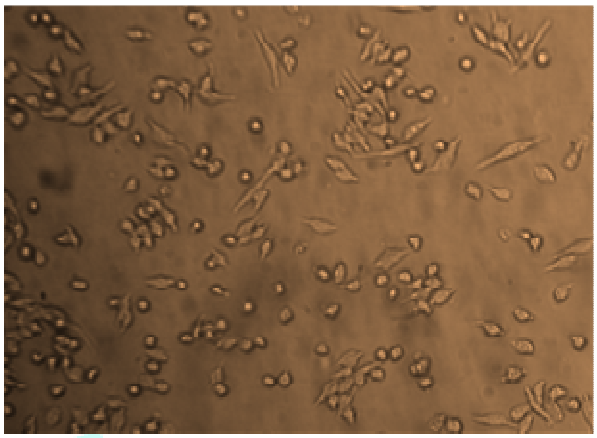

Figure 6. MTT assay on MCF-7 cancer cell line for anticancer activity of copper nanbiocomposite synthesized using leaf extract of *C. roseus*

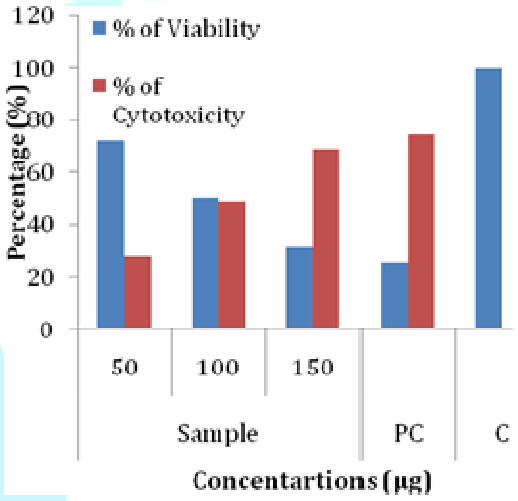

Figure 7. Cell viability and cytoxicity on MCF-7 cell line by copper nanbiocomposite synthesized using leaf extract of *C. roseus*

Conclusions

The presence of copper nanbiocomposite was confirmed by UV spectrum analysis in addition to the visual confirmation of color changed from green to greenish brown colour. The synthesized copper nanobiocomposites was in crystalline in nature with monoclinic phase. The primary and secondary amines/amides are major functional groups involved in the nanobiocomposites. The alcohols in the benzene ring are also major functional groups. The cell toxicity study confirmed that the synthesized copper nanobiocomposite can be used as an efficient anticancer agent.

Conflict of Interest

Authors declare there are no conflicts of interest.

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