In vitro cytotoxicity of copper oxide nanobiocomposites synthesized by *Catharanthus roseus* flower extract against breast cancer cell line

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ABSTRACT

Developing nanobiomaterials for therapeutic application are the latest challenge for the researchers worldwide. Nanobiomaterials synthesized by medicinal plants considered as drug carrier as well as active ingredient. These nanobiocomposites with natural active ingredient from medicinal plants reported to give better therapeutic efficacy with lesser side effects. Copper oxide nanoparticles have been reported as good antimicrobial agent. *Catharanthus roseus* is used as traditional medicine for the treatment of various diseases. Hence the present work was focused on synthesis of nanobiocomposite of copper nanoparticles using *Catharanthus roseus* flower extract. The synthesized nanobiocomposite was confirmed by UV-Vis spectroscopy analysis with maximum wave length of 363 nm. The active ingredient from flower extract bound on the surface of the copper oxide nanoparticles was studied using FT-IR spectroscopy. The crystalline nature of the synthezied nanobiocomposite was confirmed using XRD analysis. The anticancer activity of synthesized copper oxide nanobiocomposite was studied against MCF-7 cancer cell lines. The cytotoxicity of 62.96 % was observed using 150 µg of copper oxide nanobiocomposite synthesized using *Catharanthus roseus* flower extract.

KEY WORDS: Copper oxide nanoparticles, Nanobiocomposite, Characterization, *Catharanthus roseus,* anticancer activity.

1. INTRODUCTION

Nanoparticles attracted many scientists working in different disciplines due the opportunity to engineer their properties. The characteristics of nanoparticles are determined by size, shape, composition, crystallinity and morphology. Nanoparticles have distinct properties compared to bulk materials, and offer many new developments in the fields of biosensors, biomedicine and nanotechnology. Nanotechnology is used in medicine for diagnosis and as therapeutics for the treatment of diseases and disorders (Narmadha, 2013; Meenatchiammal and Vijistella, 2014). Nanotechnology is powerful technology with a promise for the design and development of many types of novel products for medical applications on early disease detection, treatment, and prevention. It is said that this field is an upcoming area of research in the modern day material science (Faraji and Wipf, 2009; Geoprincy, 2013). Nanorobots are emerging which are helpful in targeting the cancerous cells at the cellular level. Nanotechnology can be used as carriers of drug and can act as nanocarriers. It is known that most of our drugs are incapable of crossing the blood-brain barrier and hence there is always a lesser bioavailability of the drug in the body. Nanoparticles can incorporate drug into them acts as nanocarriers which can cross the blood brain barrier and thus enhancing the bio availability of the drug. The nanomedicine is specific in targeting the diseased cell forming a new method of drug delivery system (Das and Chakraborty, 2015). This means of incorporating drugs into the nanoparticles can open a new advancement in the formulation of pharmaceuticals. Copper nanoparticle is also known to be very toxic to aquatic life. Copper nanoparticls synthesis specifically has attracted more interest compared to other nanoparticles synthesis because of their useful properties achievable at much less cost than silver and gold (Zhou, 2006; Li, 2009; Chang, 2011). Copper nanoparticles exhibits good thermal and electrical conductivity, which makes it best for electronic systems and conductive inks. Similarly, it has antimicrobial properties and is readily available. These properties thus make copper nanoparticle synthesis an attractive area Xu, 1999; Masoud, 2009; Li, 2010; Wu, 2010; Usman, 2012).

Research shows that *Catharanthus roseus* contains over 130 compounds with cytotoxicity. The interest in *C. roseus* is due to therapeutic role as the source of the anticancerous alkaloids vinblastine and vincristine. Vinblastine and vincristine have very similar chemical structures, but their effects on the body are different. Vinblastine was used for the treatment of specific types of cancer including Hodgkin's disease, breast cancer, testicular cancer and lung cancer. Vincristine is used in the treatment of acute lymphoblastic leukemia. Vincristine treatment for acute lymphoblastic leukemia in children increased the survival rate to eighty percent (Heijden, 2004; Mu, 2012). Thus the present work was focused on the synthesis of nanobiocomposite of copper oxide nanoparticle using *C. roseus* flower extract and the *in-viro* anticancer activity of the synthesized nanobiocomposite was also studied against breast cancer cell line.

2. MATERIALS AND METHODS

2.1. Biological synthesis of copper nanobiocomposite using *C. roseus* flower extract: The *C. roseus* flower petals were collected and grinded with mortar and pestle using distilled water. The grinded mixture was filtered and the filtrate was collected. The mixture of 50 ml of copper sulphate and 50 ml of flower extract was heated to 80°C for 45 minutes. It was further observed for a color change from green to greenish brown for the confirmation of formation of copper nanobiocomposite. The synthesized copper nanobiocomposite was separated by centrifugation at 8000 rpm for 15 min. Then the synthezied copper nanobiocomposite was further lyophilized and stored at 4°C.

2.2. Characterization of copper nanobiocomposites synthesized using *C. roseus* flower extract: Various properties of the nanobiocomposite were studied using following characterization methods. The surface Plasmon's of the nanobiocomposite was investigated by SYSTRONICS Double beam UV- visible spectrophotometer 2201 obtaining spectrum from 300 to 800 nm. The FTIR analysis was used to characterize the functional groups of the synthesized copper nanobiocomposite using BRUKER α-T FT-IR spectrophotometer. The lyophilised samples were mixed with KBr to form a disc under high hydraulic pressure. These discs were scanned for 400 to 4000 cm-1 to obtain FTIR spectra.

2.3. Anticancer activity of copper nanobiocomposites synthesized using *C. roseus* flower extract: The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum at 37ºC in humidified atmosphere with 5% CO2.The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10^4 cells/well and allowed to attach overnight at 37ºC. The medium was then discarded and cells were incubated with different concentrations of the extracts (15, 100, 150µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

3. RESULTS AND DISCUSSION

3.1. Characterization of copper nanobiocomposite synthesized by *C. roseus* flower extract using UV spectroscopy: The reduction of the metal precursor copper sulphate was reduced by flower extract of *C. roseus*. The colloidal solution containing copper nanoparticles was changed from green to greenish brown colour of copper nanobiocomposite using flower extract. The UV absorption spectrum of copper nanobiocomposite colloid in the range of 200 to 800 nm was observed as shown Fig. 1. The existence of copper nanobiocomposite synthesized using *C. roseus* flower extract was confirmed with the peak at 387.2 nm.

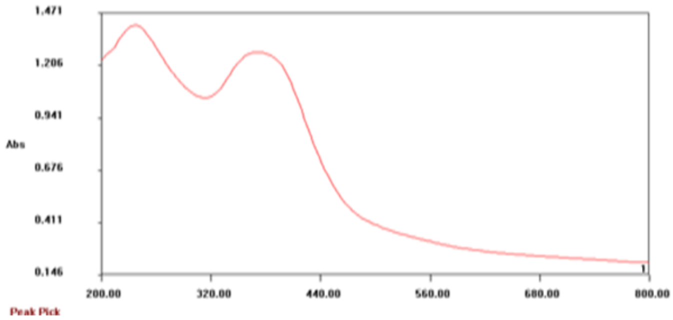

Fig.1.UV spectroscopy for copper nanbiocomposites of *C. roseus* flower extract

3.2. FTIR analysis of copper nanobiocomposite synthesized by *C. roseus* flower extract: The copper nanobiocomposite synthesized by *C. roseus* flower extract was analyzed using Fourier Transform infrared (FTIR) spectroscopy. The FTIR spectra represented an average of 50 scans. All were recorded from 400 to 4000 cm-1 as shown in Fig. 2. Sample pellet was prepared by mixing 1 mg of the lyophilized sample with 100 mg of KBr. There were 13 peaks were obtained for the copper nanobiocomposite synthesized by *C. roseus* flower extract. The peak at 3401 cm-1 showed the presence of –OH in alcohols and phenols and NH_2 in aromatic and primary amines and amides. The peak 2923 cm-1 showed the presence of CH_3 and CH_2 in aliphatic compounds. The peak 2853 cm-1 showed the presence CH_3 attached to O or N. The peak 2654 cm-1 displayed the presence of weak –CHO in aldehydes. The peak 1637 cm-1 displayed –C=O in secondary amide. The peak 1542 cm-1 showed the presence of N=N-O in azoxy compounds. The NH_3^+ in amino acids or hydrochlorides. The peak 1438 cm-1 denotes benzene ring in aromatic compounds. The peak 1225 cm-1 showed strong presence of C-N in aromatic amines. The peak 1076 cm-1 showed the presence of SO_3H in sulphonic acid. The peak 697 cm-1 denoted the presence of m-disubst.bezenes. The peak 602 cm-1 showed presence of alkyl chlorides. The peak 492 cm-1 showed very strong presence of amines, medium presence of C-O-C in ethers and NO_2 in nitro compounds.

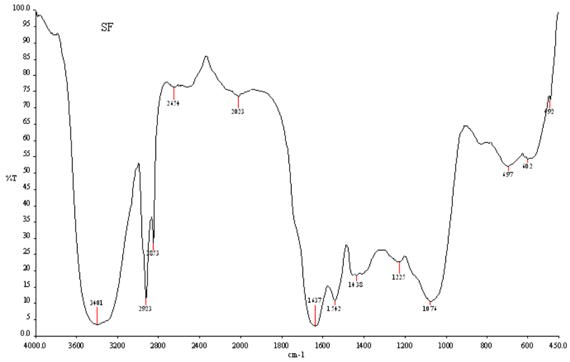

Fig.2.FTIR analysis of copper nanobiocomposite of *C. roseus* flower extract

3.3. XRD analysis copper nanobiocomposite synthesized by *C. roseus* flower extract: The XRD analysis was used to determine metallic nature and monoclinic structure of copper nanobiocomposite synthesized by *C. roseus* flower extract. For XRD analysis, the sample was prepared by centrifugation of the copper nanobiocomposite solution at 8000 rpm for 15 min. The supernatant was discarded, and the pellet was lyophilized into powder. The powder form of the copper nanobiocomposite was subjected for XRD analysis. The peak at 2θ of 35.60 in Fig. 3 confirms the crystalline nature of the synthesized copper nanobiocomposite.

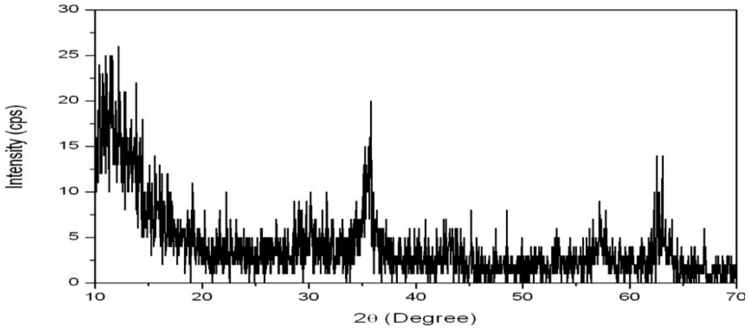

Fig.3.XRD analysis of copper nanobiocomposite of *C. roseus* flower extract

3.4. Anticancer activity of copper nanobiocomposite synthesized by *C. Roseus* flower extract against MCF-7 cancer cell line: The cytotoxicity of the synthesized copper nanobiocomposite of flower extract was studied at varied concentration of 50, 100 and 150 µg. The cytotoxicity of 21.17, 41.52 and 62.96 % were obtained for 50, 100, 150 µg respectively as shown in Fig. 4. The positive control used was cyclophosphamide which displayed 73.82 % cytotoxicity. It is observed from these results that the increase in concentration of copper nanobiocomposite has increased the cell toxicity. There is a direct proportionality between the concentration of the copper nanobiocomposite and cytotoxicity. Thus the copper nanobiocomposite synthesized by *C. roseus* flower extract can be used as an effective anticancer drug against breast cancer cells.

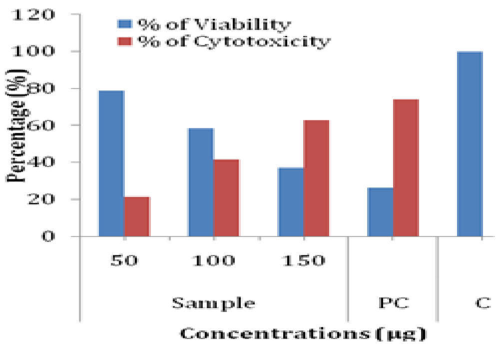

Fig.4.Cytotoxicity of copper nanobiocomposite synthesized using *C. Roseus* flower extract against breast cancer cell line using MTT assay

4. CONCLUSIONS

The visual color changed from green to greenish brown confirmed the formation of copper nanoparticles by *C. Roseus* flower extract. The XRD analysis showed that the copper nanobiocomposites provided a monoclinic phase. The FTIR results showed that primary and secondary amines/amides are major functional groups involved in the copper nanobiocomposites synthesized using *C. Roseus* flower extract. The cytotoxicity study of the copper nanobiocomposites against in-viro breast cancer cell line showed increased in cytotoxicity with increase in nanocomposite. The synthesized copper nanobiocomposites of *C. Roseus* flower extract is an efficient anticancer drug against breast cancer cells.

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