Biosynthesis of CuO NPs and its anticancer activity on human colon cancer cell lines (HT-29)

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Abstract
The environmentally friendly syntheses of the nanoparticles through the green way from the extracts of the plants has proven itself in the field of modern sciences, with improved drug efficiency and less toxicity. The study groups in the have bio-synthesized cost-effective and stable copper oxide nano-particles (CuONPs) from plant leaves (i.e. the Turmeric). A variety of the analytical approaches, like the UV-Visible Spectroscopy (UV-Vis), Fourier-Transformation Infra-red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD), have been utilized for the confirmation of the syntheses of the crystalline CuONPs from the extract of the turmeric leaves (SEM), Transmission Electron Microscopy (TEM) and Selected Area Electron Diffraction (SAED) pattern. Synthesized CuONPs have been tested as well for the anti-cancer activity with the use of the MTT (3-(4,5dimethyl-2tiazolyl)-2,5-diphenyl-2tetrazolium bromide) assay on the cell lines of the human colon cancer (HT 29). The results have shown that the synthesized CuONPs had a high anti-cancer cytotoxicity on the cell lines of the human colon cancer (HT-29) with an IC-50 value of 40 g mL⁻¹, which was briefly addressed in the present paper.

Keywords: Ecofriendly, CuO NPs, Ormocarpum cochin chinense, Cytotoxicit

1. Introduction
As compared to other synthetic medicinal substances, natural complexes that have been derived from plant materials have been considered considerably safer and easier to metabolize [1]. The synthesis of drugs is aided by secondary metabolites that have been produced from the plant materials [2]. Natural compounds account for about a quarter of all medicinal compounds used in developing countries [3].

According to the Global Cancer Report from WHO [4], cancer is a leading cause of the increased mortality rates in several nations, and it has also been regarded as a serious health threat to humanity worldwide, with new cases expected to reach about 25 million each year. The main cancer therapies, like antimetabolites, alkylating agents, and other approaches of cancer therapy, have a variety of side effects as a result of the inability to distinguish between normal and the cancer-causing cells, resulting in the toxicity. [5]. Use of the nano-materials in
Cancer care was identified as a critical component in the development of modern cancer medications [6]. NPs are often referred to as structured modern medications that are extremely beneficial in treating cancer as a result of their nano-scale sized characteristic that allows for improved drug effectiveness and long-term drug releases [7]. CuO NPs have a one-of-a-kind property in the area of the nano-scale technology. CuONPs have a narrow 1.7 eV band gap, allowing them to be used in a variety of applications such as super-conductors [8-11], solar energy conversion, syntheses of the organic as well as the inorganic nano-structure composites [12], and gas sensors [13-15]. Anti-fungal, anti-microbial, and antibiotic agents [16-17], magnetic resistant equipment [16-17], and anti-fungal, anti-microbial, and antibiotic agents [18]. Due to its biocidal properties [19-20] and anti-bacterial agent [21], CuONPs are also used in pesticide study.

Microwave irradiation [22], the sol-gel process [23], CuO NPs can be made using a variety of techniques, including electrochemistry [24], thermal de-composition [25], and an alkoxide-supported process [26]. Those methods have a number of disadvantages, including the use of hazardous materials, high energy consumption, and difficulty purifying nanoparticles [27]. Due to the toxic free chemical method in the preparation of the nano-particles, the procedure of the toxic free green material such as the plant extracts and micro-organisms [28-38] offers a variety of benefits for application in pharmaceuticals and drug discovery protection. Turmeric (L.), a member of Fabaceae family, is commonly utilized by traditional healers in the Vellore District, India, for the treatment of the disorders of the bone fractures and their related diseases [39]. The objective of the present study has been to develop a novel, toxic-free, quick, and cost-effective approach for the CuO NP synthesis using Turmeric leaf extracts.

2. Materials and Methods

Fresh O. cochinchinense leaves have been gathered in Vellore District, India (12°56'0N, 79°8'0E), and Prof. Jayaraman, PARC, India, authenticated the plant materials (No. PARC 2012-1391). Sigma Aldrich Chemicals Ltd., Mumbai, India, provided ethanol and copper chloride (CuCl2). Throughout the process, double distilled water was used experiment. For the purpose of removing filth and dust, the fresh O. cochinchinense leaves have been washed in flowing tap water. The hygienic leaf materials were dried for 72 hours in the shade at room temperature. The mechanical grinder was used to grind the dried leaf materials to fine particles. The plant materials have been obtained for 4 hours with ethanol using the Soxhlet apparatus and then undergone rotary evapometry get rid of the excess solvent. Filtered and collected condensed ethanolic leaves extract of O. cochinchinense for further processing. A mixture of 50mL freshly prepared 0.003M hydrated solution of CuCl2 and 50mL of the ethanolic leaf extracts of O. cochinchinense and continuously stirred for three hours at 60°C. UV-vis spectroscopy has been utilized for monitoring CuO NP formation. The color changes from yellow into brown due to surface plasmon resonance excitation, indicating CuONPs’ formation. Following color shift signal, The prepared material has been centrifuged for 20 minutes at 3000rpm. The pellet has been washed by the distilled water and placed in a crucible in a furnace at 400°C for two hours. The emergence of O. The presence of cochinchinense-mediated CuO NPs was verified using a variety of analytical approaches, including UV-Vis Spectroscopy (Shimadzu UV1800 PC, Japan) in the range between 200 and 800 nm [40]. The Advance Power XRD, model D 8, has been utilized for conducting the XRD study on synthesized CuONPs (Bruker, Germany). The determination of the particle size has been computed with the use of Scherrer’s equation, D = K/Cos, K represents Scherrer’s constant with a 0.94 value, is the half width limit, D stands for the particle size, represents the wave-length, and is the angle of the diffraction. FTIR Spectroscopy has been used to identify the functional group that exists in synthesized CuONPs (Bruker, Germany) from 400cm-1 to 4000cm-1 in the wave number SEM (JEOL JSM6390 LV Model) has been utilized in order to determine the form of the synthesized CuO nano-particles. The scale of CuO nano-particles mediated by O. cochinchinense synthesized was measured using a TEM (JEOL, TEM1230, U.S. (The O’s cytotoxic effects MTT assay was used to test the human colon cancer cell line (HCT116) mediated synthesized CuO NPs.
Hundreds of thousands of the cells of the colon cancer have been permitted to fix at 37°C in 96 the well plates. CuO NPs synthesized from O. cochinchinense leaves at a variety of the concentrations (10, 20, 40, 60, 80 and 100µg/L) After 24 hours, they were given cancer cells to treat. After the drug therapy, the cells have been washed in a drug-free medium. Following the completion of the time of the drug treatment incubation, 10L of 5-di-phenyltetrazolium bromide (5mg/mL in the PBS, MTT) and 10L of the 3-(4,5 dimethylthiazol-2-yl)-2 have been applied to every one of the wells, then incubated afterwards for 4 h at a temperature of 37°C, followed by 100L of 0.04mol/L hydrochloric Isopropanol contains an acid. Using an ELISA plate reader, absorbance levels have been measured at wave lengths of 570nm and 630nm for the reference as well as the test. The rate of the survival cells at each one of the different concentrations to a group and control that were utilized has been plotted[41,42].

3. Results and Discussion

Ultraviolet spectral analysis of the synthesized CuONPs have been performed on a regular basis. Converting Cu to CuONPs is mainly indicated by the shift of colour from yellow to brown. As shown in Fig. 1, ultraviolet spectrum of synthesized CuONPs clearly indicates the stability and progression. The presence of maximum absorbance at 781nm verified the nano-sized copper oxide particles’ surface plasmon resonances.

![Fig.(1): Optical absorption of CuO nanoparticles](image)

The functional groups that exist in synthesized CuONPs from the O. cochinchinense have been determined with a use of the FTIR transmittance analysis. Various characteristic peaks can be seen in the FT-IR study, 673.04cm-1, 887.59cm-1, 1383.13cm-1, 1655.89cm-1, 3424.15cm-1 in a range of 400 – 4000cm-1 (Fig2). The N-H stretch as a result of the amine group is represented by the slight broad band at 3424.15cm-1, while the peak at 1655.89cm-1 indicates the presence of C=O bending as a result of the existence of the aromatic secondary metabolites. The existence of the C-N stretch as a result of the amine group corresponds to the band at 1383.13cm-1, and the existence of =C=H bending because of group of the alkene is corresponding to peak at 887.59cm-1. The existence of the CuO vibrations in CuONPs that have been synthesized is confirmed by prominent peak at 673.04cm-1 [43].
SEM, TEM and XRD analyses of the CuONPs from O. cochinchinense XRD analyses have been performed on synthesized O. cochinchinense CuONPs for the validation of their crystalline existence (Fig. 3). The Joint Committee on Powder Diffraction Standards was involved in XRD review of the CuONPs (JCPDS), CuO NPs' crystalline existence was verified by this study (JCPDS 96-901-5925). SEM and TEM analysis were used to determine the morphological researches for synthesized O. cochinchinense mediated nano-particles of the CuO. SEM analysis of synthesized nano-particles have shown the formations of the CuO nano-particles approximately 2m and 1min, with agglomerated cluster that forms as can be seen in figure (4). CuO NP crystalline form in the agglomerated cluster structures as represented in TEM study for the synthesized nano-particles (Fig. 5). Selected Area Electron Diffraction verified crystalline nature of CuONPs that have been synthesized (SAED)

Fig(2): FTIR of CuO Nanoparticals

Fig3: XRD of CuO thin film
MTT assay has been utilized in order to investigate the cytotoxicity of Turmeric-mediated CuONPs on the cell lines or the colon cancer (HCT116). The cytotoxicity effects of O. cochinchinense mediated nano-particles of the CuO on the cell lines of the human colon cancer has been studied using a concentration range of 1g mL⁻¹, as shown in Fig6. The cytotoxicity of the synthesized copper oxide nano-particles on human colon cancer was found to be important. Fig.6 shows an example of a cell line. The cytotoxicity of the synthesized copper oxide nanoparticles on a human colon cancer cell line was demonstrated with an IC₅₀ value of 40g/mL (Fig7). When the concentration of CuO NPs has been increased, the percentage of cell viability decreased from 15% to 29%.

4.Conclusions

The results of the present work clearly show that the Turmeric leaves can be used as one of the unconventional resources for green synthesis of CuO nanoparticles. Analytical approaches such as the UV-Visible, XRD, SEM, and TEM have been used to determine the nature of synthesized CuO nanoparticles. The anticancer activity of CuONPs that have been synthesized from the Turmeric leaves was demonstrated in a human colon cancer cell line.
with an IC 50 value of 40g/mL. Furthermore, identifying and isolating the biologically active compound from Turmeric leaf extract could lead to the discovery of a new anticancer drug. The use of nano-sized CuO based drug designing molecules is largely responsible for the novel anti-cancer drug's superior efficacy and decreased toxicity.

Conflicts of Interest

There are no established conflicts of interest connected with this publication, according to the publishers.

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