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Fabrication of Curcumin-Conjugated CuO Nanoparticles and Assessment of Their Antibacterial and Anticancer Properties

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Abstract – Nanobiotechnology is a rapidly developing sector influencing every field of study, particularly therapeutic index, and drug delivery. The core of nanobiotechnology is nanoparticles, because of their incredibly tiny size and large surface area, nanoparticles are capable of bringing about treatments and cures for illnesses that were previously not achievable. In this study, curcumin copper oxide Nanoparticles were synthesized by using the precipitation method, producing a high yield of nanoparticles within less time and unexpansive chemicals that were readily available. 0.02 mol of copper acetate and 0.01 mol of curcumin were used in this research. Initially, a neon yellow color was observed which was changed into brownish black color after 1 hour stirring at 80°C and 500 rpm. UV analysis was done which gave a peak at 370nm. FTIR analysis confirmed the conjugation of Curcumin with CuO NPs and also showed phenolic, ketonic, methyl, ethyl, and Carboxyl groups were present. Agar well diffusion and culture-based antibacterial activity was performed which gave positive results against gram-negative bacteria *E.coli* and gram-positive bacteria *Bacillus subtilis*. MTT assay was performed for anticancer activity of curcumin-conjugated Copper oxide nanoparticles. Hela Cells and MTT colored reagent were used which give purple color, the intensity of color shows the cell death. The Cur-CuO NPs show 26% Cell death within 2 hours. In the future, it can be used as an anticancer drug or can also be used as a drug delivery agent.

Keywords – Nanoparticles, Curcumin, MTT assay, Anticancer agent, Drug delivery agent

Introduction

Nanobiotechnology is a revolutionary field that emerged in recent years. It involves the use of a compact version of a matter by altering its chemical or physical properties to a nanoscale between 1 and 100 nanometers. The use of nanobiotechnology has expanded from catalysts construction, and antimicrobial coatings to the detection of human illness [1] [2]. Nanoparticles exhibit size-

dependent physiochemical properties which may be attributed to their high surface-to-volume ratio. Since a large number of biological processes take place in the nanoscale range, nanomaterials are a good option for biomedical applications [3]. Nanoparticles utilized in biotechnology are between 10 and 500nm in size, rarely reaching 700nm. specifically directed to specific regions in the body after systematic distribution. Metallic nanoparticles exhibit chemical and physical features that are distinct from those of bulk metals, including unique optical properties, unique magnetizations, lower melting temperatures, mechanical strengths, and greater specific surface areas [4]. Copper oxide nanoparticles are of great interest because of their availability, stability, non-toxicity, and excellent electrical characteristics [5]. Copper oxide nanoparticles are superior to pure copper oxides exhibiting absorbance peaks ranging from 200 to 1200 nm, depending upon the kind of oxide. The copper oxide nanoparticles are stable, exhibiting a greater shelf life than most of antimicrobial drugs. Their common application lies in the medical domain where they are utilized as antimicrobial and antioxidant agents. Nanoparticles with a diameter of less than 50nm are considered promising due to heightened reactivity against microbes, large surface area, and potential for targeted delivery of antimicrobial agents [6] [7].

Materials and Method

Synthesis of copper oxide nanoparticles

In this experiment, a 0.02 mol aqueous solution of copper acetate was prepared by dissolving 0.36 grams of copper acetate in 100 ml of deionized water. The solution was heated to 70 °C while stirring at 500 rpm. After 5 minutes, 1 ml of glacial acetic acid was added, and the solution was stirred for an additional 30 minutes. Then, 1.0 gram of NaOH pellets was added to the solution, aiming for a pH of 13. This caused a color change from light blue to black, with immediate precipitation. The nanoparticles were separated by centrifugation at 8000 rpm for 5 minutes and washed with deionized water, repeating this process 3-4 times. Finally, the nanoparticles were stored in a PBS buffer for future use.

Synthesis of Curcumin conjugated copper oxide nanoparticles

For the synthesis of curcumin conjugated copper oxide nano particles, a copper acetate solution (0.02 mol) is prepared, and glacial acetic acid is added after 5 minutes of stirring at 70°C. A curcumin solution (0.01 mol) is prepared by mixing curcumin with ethanol and NaOH, and it is added to the copper acetate solution along with 40% PEG. The mixture is heated and stirred for 30-40 minutes. NaOH is added to adjust the pH to 13, resulting in the immediate formation of

brown-black precipitation. The solution is cooled and then centrifuged for 10 minutes at 8000 rpm. The nanoparticles are washed multiple times with deionized water. Finally, the nanoparticles are suspended in PBS buffer and stored at 4°C for future use. Curcumin is used as conjugating agent whereas PEG is used as a surfactant to synthesize conjugated nanoparticles from copper acetate. The addition of NaOH adjusts the pH and induces nanoparticle formation, which is then separated, washed, and stored for further applications.

Characterization of CuO – NPs

Characterization of the synthesized nanoparticles was performed to confirm their properties and composition using various techniques. A change in solution color served as an initial indication of nanoparticle formation. X-ray diffraction (XRD) analysis was conducted on air-dried, powdered samples stored in Eppendorf tubes to determine structural properties. FTIR analysis was used to identify the elemental composition, and functional groups present in the nanoparticles. UV-visible spectroscopy, performed using a 10x diluted solution, revealed absorbance peaks for CuO nanoparticles in the range of 250–350 nm.

Agar Well Diffusion Method

First step is to prepared inoculum. Broth was prepared and placed into the test tube, autoclaved the test tube and picked a colony from a culture media with the help of sterilized loop and shake the loop into the broth. Incubated the broth into shaking incubator at 37°C for 16 hours (overnight). On the next day cloudy solution appeared in the broth and showed bacterial growth. For agar well diffusion method, agar was prepared, autoclaved the solution and poured the thin layer of agar into petri dish and dried into laminar hood. 20 µl of bacterial culture was added on a dried plate and spread it with the help of sterilize spreader. Wells was created with help of cork bore or micropipette tip. Labeled each well to the volume of nanoparticles was loaded. 20µl, 40µl, 60µl and 80µl of nanoparticles sample is loaded. Plates was covered and incubated it at 37°C for 16-17 hours (overnight). Repeat the process for each bacterial strain. Next day inhibition zone was measured.

Cell Culture Based Method

For culture-based method, inoculate the bacterial culture. 4 test tube is required for this method each test tube must be autoclaved. First step was to prepare the inoculum, filled one test tube

with broth and autoclave the test tube. Picked a colony of desire bacteria with help of sterilize loop and shake into broth. Placed it into shaking incubator at 37°C for 16 hours. On the next day cloudy appearance showed in the test tube broth. Transfer 3ml inoculum in each autoclaved test tube and labeled it with control, 100µl, 200µl and 300µl. Transfer nanoparticles sample in each labeled test tube, respectively. Placed the inoculum with loaded sample into shaking incubator for 3-4 hours. Checked the OD of all samples at 600nm and recorded the reading.

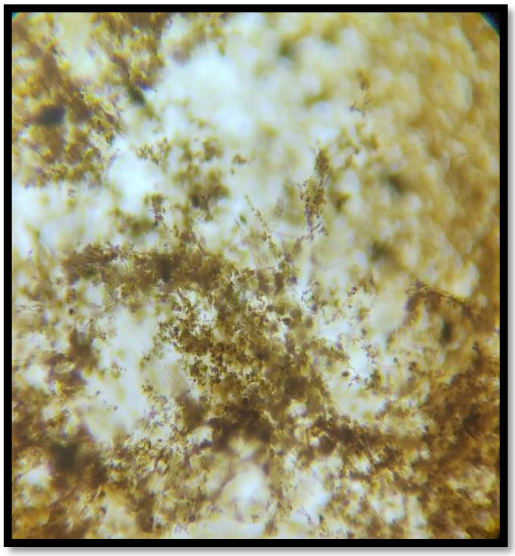
MTT Assay

MTT assay is performed for anticancer property of Cur-Cu NPs for that 1000 Hela cells were required and that was plated in 24 well plate. Incubated the well for 24 hours, one well as control and one well with nanocomposite. After 24 hours 10µl MTT reagents (12mM) is added in both wells and incubated for 2-4 hours in carbon dioxide incubator. After 4 hours of incubation 100µl of detergent (10g 0.01M HCl and 1g SDS) is added in both wells and incubated the sample in a dark place at a room temperature for further 2 hours. At 570nm measured the absorbance of both samples.

Results

Synthesis of Cur-CuO NPs

The Cur-CuO NPs were prepared using curcumin as conjugating agent and PEG as surfactant to synthesize conjugated NPs from copper acetate. The addition of NaOH adjusts the pH and induces nanoparticle formation, which is then separated, washed, and stored for further applications.



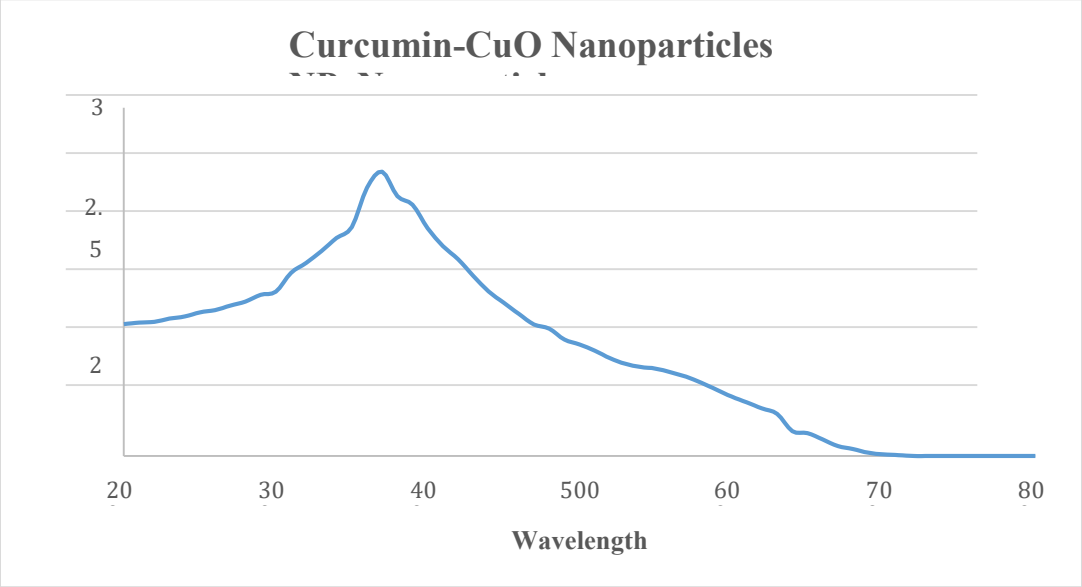
(a) 4x Zoom

(b) 10x Zoom

Figure#1 Microscopic images of Cur-CuO NPs

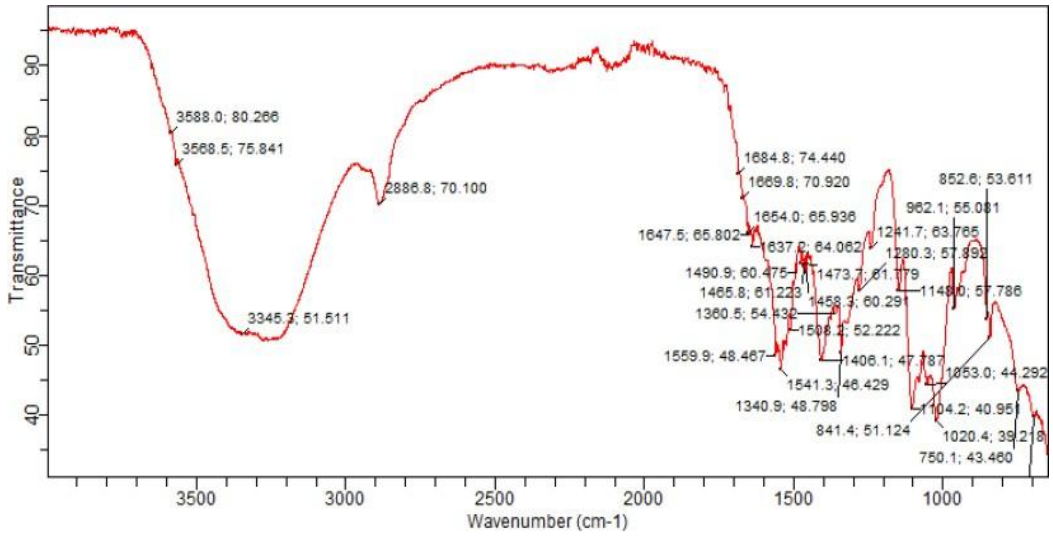
Characterization of Cur-CuO NPs

The confirmatory analysis for Cur – CuO NPs was done by X-ray diffraction studies (XRD), further supported by FTIR and UV-visible spectroscopy. UV analysis was performed after every 30 minute of time interval the shifting of peak absorbance confirms the conjugation and recorded the highest peak absorbance. UV analysis for Curcumin-CuO nanoparticles showed absorbance peak at 370nm as shown in the graph (Figure 1.1.2).



Figure#2 UV analysis of Curcumin-CuO NPs

Further characterization was carried out by FTIR. It is the confirmatory test to observe the capping of curcumin with copper oxide nanoparticles. The wavenumber range is 650 to 4000 cm^{1}. The wavenumber range 4000 to 2500 cm^{-1} revealed the single bond stretch, 2500to 2000 cm^{-1} range revealed nitriles carbine triple group, 2000 to 1500 cm^{-1} range revealed the double groups and 1500 to 500 cm- 1 revealed fingerprint region single bonds. The FTIR results show that the curcumin is capped with copper oxide nanoparticles. As that can be compared in the graph the peaks in 1700 to 600 cm^{-1} range is high that indicates the conjugation of curcumin with CuO NPs.



Figure#3 FTIR Analysis of Cur-CuO Nanoparticles

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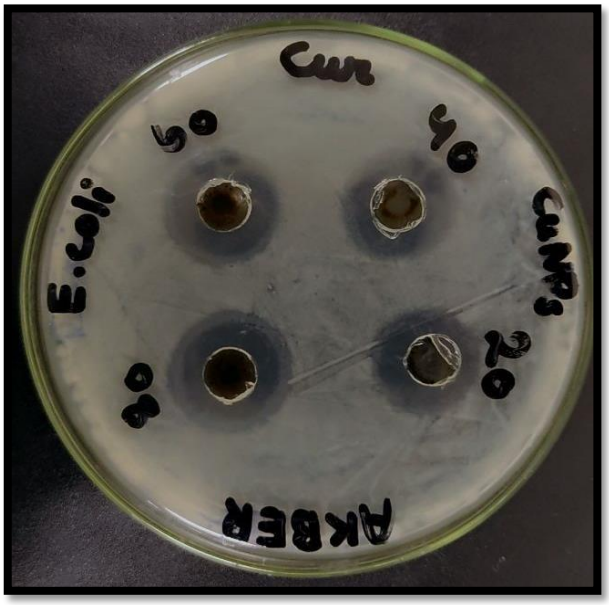
Antibacterial properties

Agar well diffusion method

Agar well diffusion method was used to check the antibacterial property of synthesized nanoparticles. This activity was performed against the gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *E. coli.* Curcumin-CuO nanoparticles showed positive results. After 16 hours of incubation clear inhibition zone was observed and measured.



Figure#4 Cur-CuO NPs activity against *E. coli*

Figure#5 Activity against *B. subtilis*

Culture Based Method

Culture based method is used to check the antibacterial activity in suspension solution. Same gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *E.coli* were used. 3-4 hours of incubation with nanocomposite showed positive results as shown in the figures. Without shaking the test tube picked the sample and then checked optical density at 600nm.

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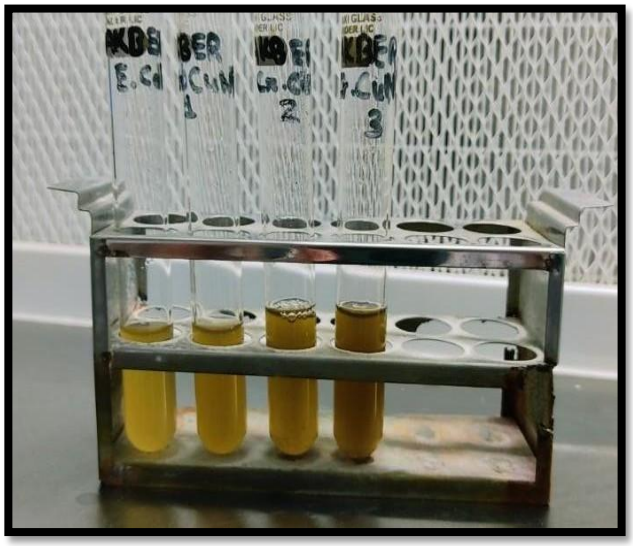

Figure #5 Culture based activity against *E. coli*

Figure #6 Cultures based activity against Bacillus subtilis

MTT Assay

MTT proliferation assay was performed to study the effects of Cur-CuO NPs on the HeLa cell line. After incubation in a dark for two hours, Cur-CuO NPs showed positive results against Hela cells. Color change can visibly be observed after addition of MTT reagent and detergent reagent. Higher Color intensity represent live cells present in culture. Checked absorbance of control and test sample at 570 nm. Absorbance of control at 570nm was 1.754 whereas, absorbance of test at 570nm was 1.587. The test sample showed less absorbance which indicates decrease in the synthesis of NADP that mean cell proliferation decreases after adding the Cur-CuO NPs. Which indicates cell death occurs in addition of Cur-CuO NPs while control sample showed cell proliferation.

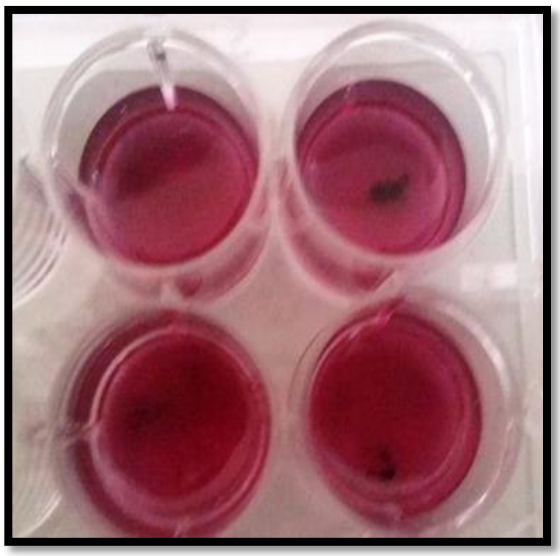

Figure #7 MTT colored Product

Discussion

The current research focuses on the synthesis, characterization, and application of curcumin-conjugated copper oxide (CuO) nanoparticles [8]. The synthesis process employed an inexpensive extraction method, wherein the chemical components in the solution acted as significant reducing agents [9]. Notably, no stabilizing agents, such as cetyltrimethylammonium bromide (CTAB), were utilized during this process [10]. Instead, sodium hydroxide (NaOH) served as an effective stabilizing agent, simplifying the synthesis pathway. The synthesized CuO nanoparticles exhibited excellent catalytic properties, particularly in the reduction of methylene blue. Among the various morphologies, nanograins demonstrated superior catalytic activity compared to spherical nanoparticles, underscoring the critical role of particle shape in determining catalytic efficiency. The nanoparticles were characterized by a sharp absorption peak at 370 nm, which confirmed the complete reaction and conjugation of curcumin with CuO nanoparticles. This peak served as clear evidence of successful nanoparticle synthesis [11].The study further explored the biological applications of these nanoparticles. Antibacterial activity tests conducted against *Escherichia coli* and *Bacillus subtilis* showed positive results, demonstrating the nanoparticles' effectiveness in combating bacterial strains. Additionally, the anticancer potential was evaluated using the MTT assay, which revealed that the curcumin-conjugated CuO nanoparticles effectively inhibited the proliferation of HeLa cells. These findings highlight the multifunctionality of Cur-CuO nanoparticles, particularly in catalysis, antibacterial treatments, and cancer therapy.

Conclusion

Drug delivery utilizing nanoparticles is appreciated as a promising and effective method for enhancement of safety curcumin. The basic aim of study was to evaluate the efficiency of Cur-CuO NPs as a potential anticancer and antibacterial agent. The curcumin conjugated copper oxide nanoparticles were synthesized by precipitation method. Characterization was by using FTIR and UV spectrophotometer, antibacterial and anticancer activity of curcumin copper oxide nanoparticles were studied. In our study, the conjugation of curcumin with copper oxide nanoparticles was confirmed by FTIR and by using UV Spectrophotometer that showed the peak range was 340 to 370nm. Antibacterial activity showed positive results of Cur-CuO NPs. Anticancer activity showed positive results which highlights that it can be used as anticancer drug or as drug delivery agent. Our results signify the importance of curcumin as a promising antiangiogenic and anticancer agent. Nanobiotechnology is constantly expanding in terms of biomedical applications. Hence, there is need to critically evaluate such bioactive molecules.

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