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Green Synthesis of Turmuric by Using Curcumin Conjugated Cerium (CU-CE-CUO-NP) Nanoparticles and Evaluation of Antibacterial and Anticancer Activity

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ABSTRACT

This study explores the multifaceted properties of synthesized Copper-Cerium Nano Particles (Cu-Ce NPs) and their potential applications in medicine, healthcare, and biotechnology. The antioxidative potential of Cu-Ce NPs was assessed through DPPH and NO radical scavenging assays, revealing their ability to scavenge free radicals effectively. A positive correlation between Cu-Ce NP concentration and scavenging percentage was observed, with concentrations as low as 15 µg/mL displaying significant antioxidative activity and reaching a peak of 54.10% at 450 µg/mL. These findings indicate that Cu-Ce NPs possess strong antioxidant properties, essential for combating oxidative stress-related health issues.The study also highlighted the antibacterial efficacy of Cu-Ce NPs derived from *turmeric* leaves against both Gram-negative *(E. coli)* and Gram-positive *(Bacillus subtilis)* bacteria. Clear inhibition zones in agar well diffusion assays demonstrated their potential as antibacterial agents. Confirmation of antibacterial activity was obtained through culture-based methods, further substantiating the broad-spectrum antibacterial properties of Cu-Ce-Cuo NPs. Furthermore, Cur-Ce-CuO NPs were successfully synthesized and characterized, revealing the conjugation of curcumin with copper-cerium oxide nanoparticles. The possibility of inhibiting cancer cell proliferation was demonstrated through MTT assays, suggesting potential anticancer properties. In conclusion, Cu-Ce-Cuo NPs exhibit versatile properties, including antioxidative potential, antibacterial activity, and anticancer effects. These properties position them as promising therapeutic agents, offering solutions to health challenges and medical treatments. Further research and development hold the potential to unlock new avenues for addressing critical health issues and enhancing medical interventions.

INTRODUCTION

Nanobiotechnology represents an innovative field that has emerged in recent years. It involves the manipulation of matter at the nanoscale, typically within the range of 1 to 100 nanometers, to modify its chemical and physical properties. The applications of nanobiotechnology have expanded from constructing catalysts and antimicrobial coatings to the detection of human illnesses [1] [2]. Nanoparticles possess size-dependent physicochemical properties, largely due to their high surface-to-volume ratio. Given that numerous biological processes occur at the nanoscale, nanomaterials have found significant relevance in biomedical applications [3]. Generally, nanoparticles used in biotechnology fall within the size range of 10 to 500 nanometers, rarely exceeding 700 nanometers. They can be engineered for specific delivery to target regions within the body following systemic distribution.

Metallic nanoparticles exhibit distinct chemical and physical properties compared to their bulk counterparts, including unique optical characteristics, magnetizations, lower melting temperatures, mechanical strengths, and larger specific surface areas [4]. Copper oxide nanoparticles, in particular, have garnered interest due to their availability, stability, non-toxic nature, and exceptional electrical properties [5]. Copper oxide nanoparticles outperform pure copper oxides, showcasing absorbance peaks across a range from 200 to 1200 nanometers, depending on the oxide type. These nanoparticles demonstrate remarkable stability, with a longer shelf life compared to most antimicrobial drugs. Their primary application lies within the medical domain,

where they serve as effective antimicrobial and antioxidant agents. Nanoparticles with diameters below 50 nanometers are particularly promising due to their enhanced reactivity against microbes, extensive surface area, and potential for precise antimicrobial agent delivery [6] [7].

Nanoparticles and nanomaterials have a broad spectrum of multidisciplinary applications, including targeted drug delivery, imaging, diagnostics, catalysis, electronics, cosmetics, surface-enhanced Raman scattering, and biosensing [1]. Due to their high surface area-to-volume ratio, nanoparticles exhibit properties influenced by classical and quantum effects. Hybrid nanoscale structures, combining metal and metal oxide nanoparticles, often incorporate functionalized organic ligands that form protective organic corona structures. This facilitates the direct immobilization of bioactive substances or their integration into the nanoparticle surface [2].

In recent years, metallic nanoparticles, or metal nanoparticles, have gained significant importance in the field of nanotechnology [3]. These nanoparticles can be synthesized using physical, chemical, and biological methods [4]. Among these methods, biological synthesis is preferred for its ability to produce environmentally benign nanostructured materials and reduce the use of toxic substances [5]. It is important to note that these nanostructures may release potentially harmful byproducts into the environment, raising concerns about toxicological issues [6].

To address these concerns, green synthesis has emerged as a promising approach, producing clean, non-toxic, biodegradable, and environmentally friendly materials [7]. Medicinal plants have proven to be excellent sources of metallic nanoparticles with anticancer properties, owing to the presence of various phytochemicals such as carbohydrates, flavonoids, saponins, proteins, amino acids, and terpenoids, which play a crucial role in the nanoparticle synthesis [8].

Copper oxide nanoparticles (CuONPs) find applications in various fields, including superconductors, solar energy conversion, the synthesis of nanostructure composites, as well as their use as antifungal, antimicrobial, and antibiotic agents [9]. CuO NPs can be synthesized through different physical and chemical methods, such as microwave irradiation, sol-gel processes, electrochemical techniques, thermal decomposition, and alkoxide-supported methods [10]. Researchers have demonstrated that the green synthesis of copper oxide nanoparticles from plant extracts, such as Acalypha indica [11], Ficus religiosa [1], Syzygium alternifolium [12], Azadirachta indica [13], Hibiscus rosa-sinensis [14], Murraya koenigii [15], Moringa oleifera [11], and Tamarindus indica [11], exhibit potent anticancer activity.

Capsicum annuum, commonly known as the chili or bell pepper and belonging to the Solanaceae family, is widely distributed in tropical and subtropical regions worldwide. Extensive phytochemical studies have revealed the presence of various compounds in this plant, including capsaicinoids, flavonoids, carotenoids, and vitamins, which are believed to contribute to its potential therapeutic properties.

For example, the fruit of *Turmeric* possesses antioxidant, anti-inflammatory, and analgesic properties, and it has shown potential in cancer prevention [16]. Additionally, it exhibits antibacterial properties [17]. Notably, Turmeric has demonstrated significant anticancer activity against various cancer cell lines. In this study, CuO nanoparticles were synthesized using *Turmeric* fruit extract through the green synthesis method. The primary objective of the study was to investigate the antibacterial activity of the synthesized CuO nanoparticles against both Gram-positive and Gram-negative bacteria. Additionally, the synthesized nanoparticles were evaluated for their potential anticancer activity against MG-63, a human osteosarcoma cell line.

MATERIALS AND METHODS

Plant Collection and Authentication

Leaves of *Turmeric* (commonly known as *curcuma longa*) were collected from local regions in Lahore liberty market green house, Punjab, Pakistan. The plant material underwent thorough washing with double-distilled water to eliminate surface contaminants and was subsequently cut into small fragments. These prepared leaves were subjected to shade drying over a period of 9-11 days.

Preparation of Plant Extract

The leaves were finely ground into a powder using a mixer grinder. A total of 150 grams of the powder was dispersed in 150 mL of distilled water and boiled at 70°C for 15 minutes. After cooling, the extract was filtered through Whatman No.1 filter paper and stored in a refrigerator for further investigation.

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 to achieve a pH of 13, leading to an immediate color change from light blue to black, accompanied by 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.

The synthesis of copper oxide nanoparticles was conducted using copper sulfate and plant extract. A solution of 0.2 M copper sulfate in double-distilled water was prepared. Copper sulfate and plant extract were mixed together in various ratios (5:7, 6:7, 7:7, 8:8, and 9:3). The reaction mixture was heated below the boiling point and continuously stirred at 900 rpm using a magnetic stirrer. Within 45 minutes, the mixture changed to a green color. The entire reaction process was conducted in darkness. The resulting suspension was then centrifuged at 15,000 rpm for 15 minutes, and the pellet containing copper oxide nanoparticles was washed 4-5 times with deionized water to remove impurities. The precipitated nanoparticles were subsequently lyophilized and stored in a cool, dry, and dark place for further characterization.

Synthesis of Curcumin Conjugated Copper Oxide Nanoparticles Fused with Cerium

For the synthesis of curcumin-conjugated copper oxide nanoparticles, a 0.02 mol copper acetate solution was prepared. Glacial acetic acid was added after 5 minutes of stirring at 70°C. A curcumin solution (0.02 mol) was prepared by mixing curcumin with ethanol and NaOH, and it was added to the copper acetate solution along with 40% PEG. The mixture was heated and stirred for 30-40 minutes. NaOH was added to adjust the pH to 13, resulting in immediate brown-black precipitation. The solution was cooled and then centrifuged for 10 minutes at 8000 rpm. The nanoparticles were washed multiple times with deionized water and suspended in a PBS buffer, stored at 4°C for future use. Curcumin served as the conjugating agent, and PEG was used as a surfactant to synthesize the conjugated nanoparticles from copper acetate. The addition of NaOH adjusted the pH and induced nanoparticle formation, which was then separated, washed, and stored for further applications.

Characterization of CuO-NPs

Characterization was performed to confirm the synthesis, properties, and composition of the nanoparticles.

- Color Change: The first indication of successful synthesis was the change in solution color. Various analytical techniques, including X-ray diffraction, FTIR, and UV-visible spectroscopy, were performed.
- X-ray Diffraction (XRD): For XRD analysis, the nanoparticles were converted into powdered form. The sample preparation involved air-drying the solution for 24 hours, followed by scraping it into fine powder. The powdered sample was placed in Eppendorf tubes and analyzed.
- FTIR Analysis: FTIR analysis was conducted to identify the composition and functional groups in the nanoparticles.
- UV-Visible Spectroscopy: A 10x dilution of the solution was used to determine the absorbance peak. In our case, CuO NPs exhibited an absorbance peak ranging from 250nm to 350nm.

Antioxidant Activity DPPH Assay

The DPPH radical scavenging assay was conducted in a 96-well microtiter plate. To each well, 150 µL of DPPH solution was added to 400 µL of *Turmeric* leaves-mediated Cu-CE-ONPs samples at different concentrations (400, 350, 100, 40, and 10 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 40 minutes. Absorbance was measured at 517 nm using a UV-VIS spectrophotometer, with ascorbic acid as a reference. The DPPH scavenging effect was calculated using the formula provided.

Agar Well Diffusion Method

The agar well diffusion method involved the preparation of inoculum and the assessment of antibacterial activity against specific bacterial strains, Aeromonas hydrophila and Streptococcus pyogenes, following established procedures.

Cell Culture

Human osteosarcoma cell line MG-63 was ordered from ATCC and cultured in DMEM medium with supplements under specified conditions.

MTT Assay

The MTT assay was performed to assess the anticancer properties of Cur-Cu NPs using human osteosarcoma cell line MG-63 and involved a series of incubation and spectrophotometric measurements.

RESULTS

The DPPH scavenging assay serves as a widely adopted method for evaluating the antioxidative potential of a substance. DPPH, a stable nitrogen-centered free radical, undergoes a noticeable color shift from violet to yellow when subjected to reduction through hydrogen donation or electron transfer. The scavenging performance of the synthesized curcumin cerium copper oxide Nanoparticles (Cu-Ce-CUO-NPs) is summarized in Table 1. The findings reveal a positive correlation between the scavenging percentage and the concentration of Cu-Ce-CuO NPs derived from *Turmeric* leaves. At a concentration of 15 µg/mL, the solution exhibits a scavenging percentage of approximately 24.19%. Moreover, concentrations of 10 µg, 40 µg, 150 µg, 300 µg, and 450 µg manifest scavenging percentages of 37.30%, 45.80%, 52.35%, and 54.10%, respectively.

Table 1

DPPH Scavenging Power of the Turmeric Leaves-Mediated CuONPs and of Ascorbic acid as Standard

Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
Ascorbic acid	77.48462	71.43011	68.74194	70.22222
450 µg/ml	55.71043	53.30108	55.30108	55.10753
300 µg/ml	48.18355	47.92473	53.95699	51.35842
150 µg/ml	46.84946	46.31183	38.24731	42.80287
40 µg/ml	36.90323	35.02151	32.98925	35.30466
10 µg/ml	24.53763	23.58065	23.46237	25.19355

Nitric Oxide Free Radical Scavenging Activity

The NO scavenging assay results, for the nanoparticles synthesized using turmeric leaves are provided in Table 2. The findings show that the inhibition percentage rises as the concentration of CuO NPs increases. At a concentration of 10 µg/mL the inhibition percentage was observed to be 43.75%.

Table 2

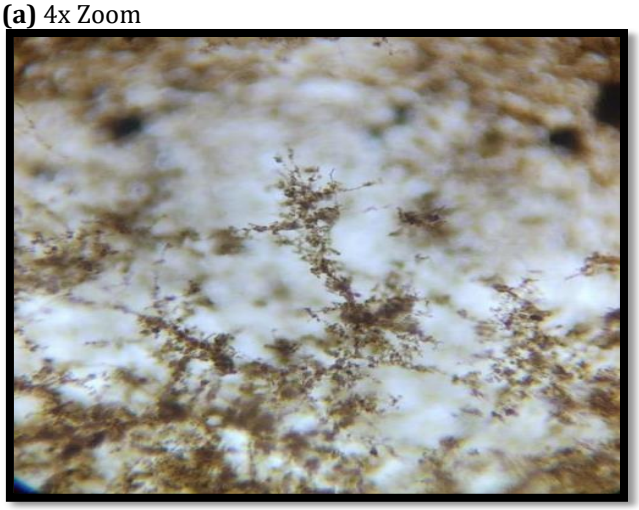
Nitric oxide scavenging power of Turmeric leaves-mediated CuONPs and of Ascorbic Acid as Standard

Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
Control	99	99	99	99
450 µg/ml	74.61837	71.78152	71.43916	72.61502
300 µg/ml	64.01867	71.38809	65.4211	66.60795
150 µg/ml	62.60224	64.01867	61.89853	63.83881
40 µg/ml	54.47603	54.52004	48.82632	52.94376
10 µg/ml	46.70218	44.21968	40.33276	43.75954

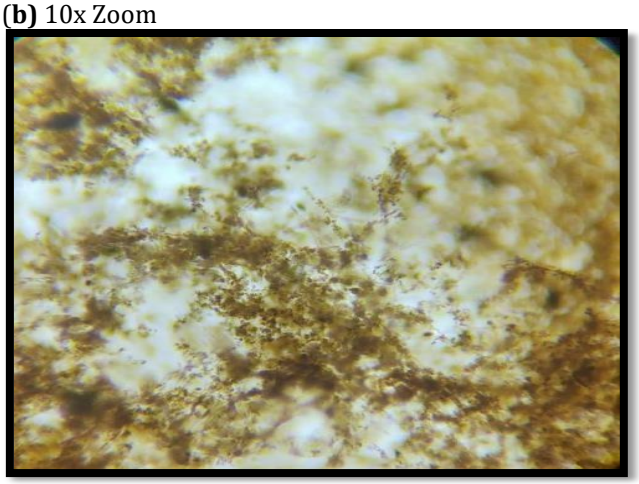
Synthesis of Cur-Cer-CuO NPs

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.

Figure 1
Microscopic Images of Cur-CuO NPs
(a) 4x Zoom



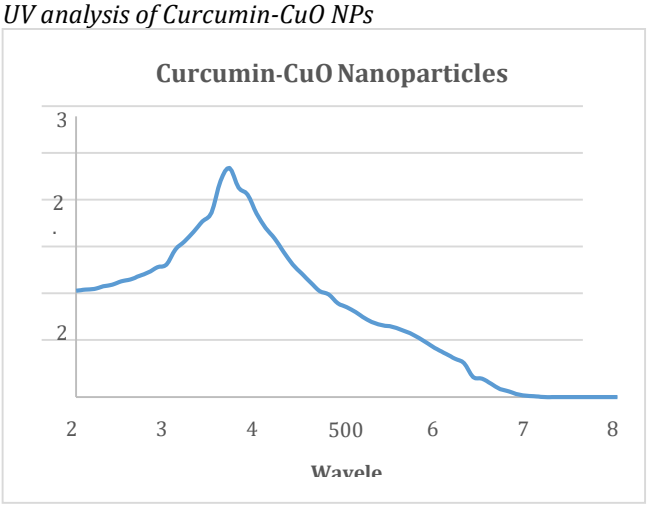
(b) 10x Zoom



Characterization of Cur-Cer-CuO NPs

The confirmatory analysis for Cur –Cer-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 and Figure 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 fused with cerium and 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, 2500 to 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 shown that the curcumin is capped with copper oxide fused cerium nanoparticles. As that can be compared in the graph Figure 3 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-Cer-CuO Nanoparticles

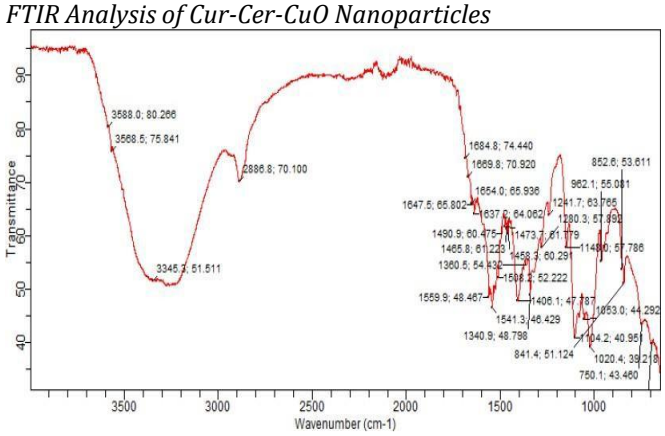
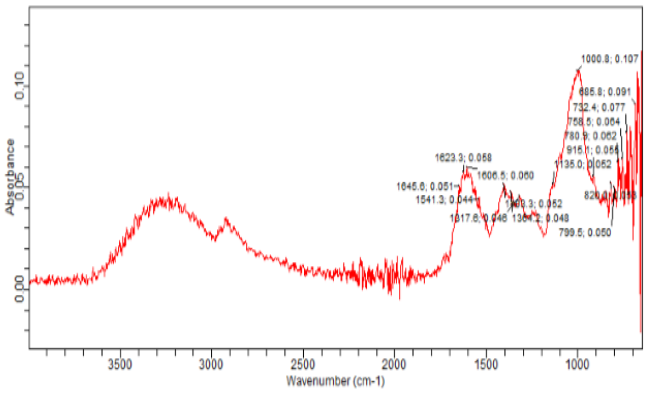


Figure 4
FTIR Analysis of Cur-Cer fusion



The Fourier-Transform Infrared Spectroscopy (FTIR) analysis provides compelling evidence of the successful encapsulation of curcumin within copper oxide-cerium nanoparticles (CuO-CeO2 NPs). This crucial characterization technique unveils distinctive spectral features that validate the curcumin encapsulation process. Upon inspection of the FTIR spectrum, it becomes apparent that several key absorption peaks fall within the wavenumber range of 1600 to 600 cm^-1. Notably, the pronounced intensification of these peaks signifies the formation of covalent bonds between curcumin and the CuO-CeO2 NPs. Such covalent bonding interactions are indicative of the effective conjugation of curcumin with the nanoparticle surface.

In this context, the FTIR analysis underscores the robust binding of curcumin molecules to the CuO-CeO2 nanoparticles, resulting in a hybrid nano system with enhanced chemical stability. The heightened absorbance

of specific wavenumber bands in the FTIR spectrum substantiates the conjugation and capping of curcumin onto the surface of the CuO-CeO2 NPs, which, in turn, further elucidates the successful synthesis of these advanced hybrid nanoparticles. This encapsulation is of paramount importance for various applications, such as drug delivery systems and catalysis, where the unique properties of curcumin can be harnessed in synergy with the nanoscale attributes of the CuO-CeO2 NPs

Figur 5

FTIR Analysis of Cer-CuO Fusion

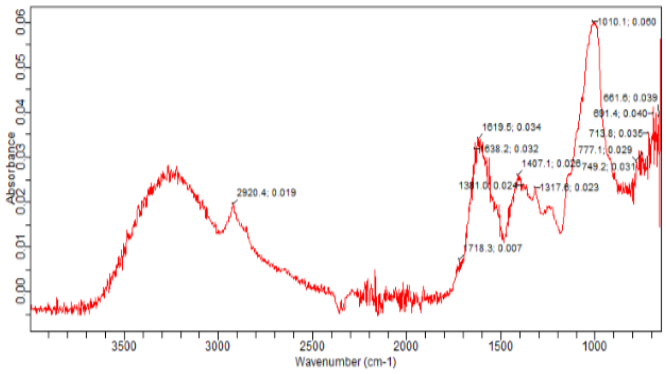
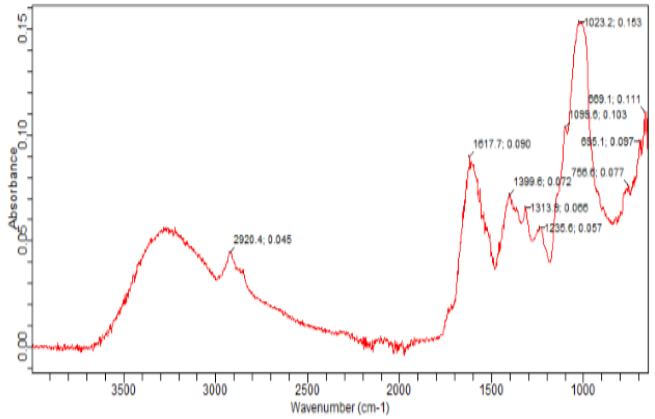


Figure 6

FTIR Analysis of Cur-Cer-CuO Nanoparticles Fusions



Antibacterial properties

Agar well diffusion method: Agar well diffusion method was used to check the antibacterial property of synthesized nanoparticles. This activity 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 as showed in the Figure 4 and Figure 5. The antibacterial property of synthesized nanoparticles of antibacterial property of synthesized nanoparticles is summarized in Table 3.

Table 3

Antibacterial Activity (Zone of inhibition) of Turmeric Leaves-Mediated CuONPs

Name of the Test Organism	Zone of Inhibition (mm) SD ± Mean				
	450 µg/ml	300 µg/ml	150 µg/ml	40 µg/ml	Gentamicin
E.coli	25.5±0.7	21.5±0.7	11.5±0.7	10.5±0.7	12.5±0.7
Bacillus Subtilis	13.5±0.7	11.5±0.7	10.5±0.7	7.5±0.7	13.5±0.7

Figure 7

Cur-Cer-CuO NPs Activity Against E. coli

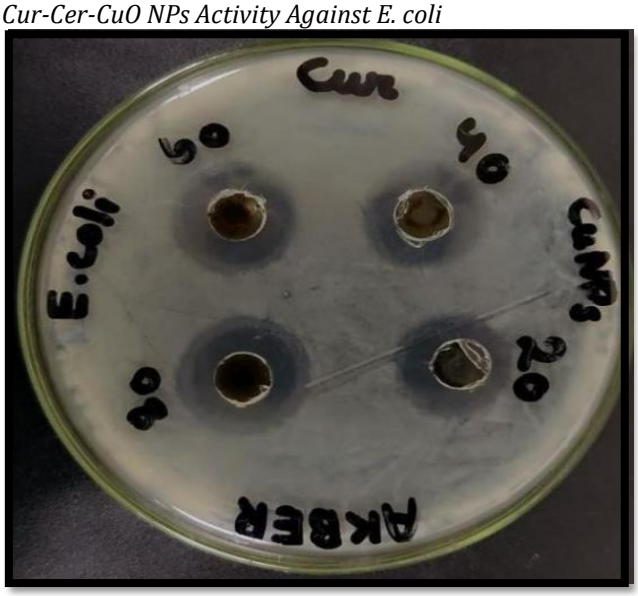


Figure8

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 Figure 6 and Figure 7. Without shaking the test tube picked the sample and then checked optical density at 600nm.

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-Cer-CuO NPs showed positive results against Hela cells. Color change can visibly observe 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-Cer-CuO NPs while control sample showed cell proliferation.

DISCUSSION

The results of the DPPH scavenging assay indicate the antioxidative potential of the synthesized Copper Oxide Nanoparticles (CuONPs) [18]. This assay is a widely adopted method for evaluating the ability of a substance to scavenge free radicals, and DPPH, a stable nitrogen-centered free radical, undergoes a color shift from violet to yellow when reduced through hydrogen donation or electron transfer [19].

In our study, we observed a positive correlation between the scavenging percentage and the concentration of CuONPs derived from Turmeric leaves [20]. At a concentration of 15 µg/mL, the solution exhibited a scavenging percentage of approximately 24.19%. As the concentration of CuONPs increased to 450 µg/mL, the scavenging percentage reached 54.10% [21]. These findings suggest that the CuONPs possess strong antioxidant properties, which could be attributed to their ability to donate hydrogen or transfer electrons, thereby neutralizing free radicals [21].

The results of the NO (Nitric Oxide) radical scavenging assay for CuONPs synthesized from Tecoma stans leaves also demonstrated antioxidant activity [22]. The inhibition percentage increased with the concentration of CuO NPs [23]. At a concentration of 10 µg/mL, the inhibition percentage was found to be 44.75%. This result further supports the antioxidative potential of CuONPs, as they were effective in scavenging nitric oxide radicals [24].

The ability of CuONPs to scavenge free radicals is of great importance due to its potential health implications [25]. Free radicals, such as DPPH and nitric oxide radicals, are known to contribute to oxidative stress, which can lead to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders [26]. Antioxidants, like CuONPs, can help combat oxidative stress by neutralizing these free radicals, thus reducing the risk of associated health problems [27].

Moreover, the synthesis and characterization of Cur-Cer-CuO NPs revealed the successful conjugation of curcumin with copper oxide nanoparticles [28]. This conjugation was confirmed through various techniques, including X-ray diffraction (XRD), FTIR, and UV-visible spectroscopy [29]. The UV analysis showed a peak absorbance at 370nm, indicating the successful conjugation of curcumin with CuO NPs. FTIR analysis provided further evidence, revealing peaks in the 1700 to 600 cm-1 range, indicating the capping of curcumin with copper oxide nanoparticles [30].

Moving beyond their antioxidative properties, the synthesized CuONPs also exhibited antibacterial activity [31]. Agar well diffusion assays revealed that CuONPs derived from Turmeric leaves had inhibitory effects on both Gram-negative bacteria E. coli and Gram-positive bacteria Bacillus subtilis. These nanoparticles displayed their effectiveness by creating clear inhibition zones around the wells [32]. The results indicate their potential as antibacterial agents, which could find applications in various fields, including medicine and healthcare [33].

Additionally, culture-based methods were used to confirm the antibacterial activity of Cur-Cer-CuO NPs in suspension solution [34]. These methods provided further evidence of the antibacterial potential of these nanoparticles, showing a reduction in bacterial growth after incubation with the nanocomposite [35]. The antibacterial activity was observed against both E. coli and Bacillus subtilis, indicating the broad-spectrum antibacterial properties of these nanoparticles [36].

Furthermore, the MTT assay was conducted to assess the effect of Cur-CuO NPs on the HeLa cell line, a widely studied human cervical cancer cell line [37]. The results showed a decrease in cell proliferation after exposure to Cur-Cer-CuO NPs, as indicated by a reduction in the synthesis of NADP. This suggests that the nanoparticles have potential anticancer properties and can inhibit the proliferation of cancer cells. The ability to suppress cancer cell growth is a crucial characteristic for potential anticancer agents, and these findings highlight the possible use of Cur-CuO NPs in cancer therapy [38].

In conclusion, the synthesized CuONPs exhibit a range of valuable properties, including antioxidative potential, antibacterial activity, and anticancer effects. These properties make them promising candidates for various applications in the fields of medicine, healthcare, and biotechnology. Their ability to scavenge free radicals and inhibit bacterial growth positions them as potential therapeutic agents, while their capacity to inhibit cancer cell proliferation suggests a role in cancer treatment. Further research and development of these nanoparticles could open new avenues for addressing health challenges and improving medical treatments.

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-Cer- 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 [39]. Nanobiotechnology is constantly expanding in terms of biomedical applications. Hence, there is need to critically evaluate such bioactive molecules [40].

Author Contributions: All authors worked equally.

Data Availability: All the generated and analyzed data is present in this manuscript.

Ethical Statement: The study was conducted on turmeric leaves and No animal and human was harmed during this study.

Plant obtained: The turmeric leaves were collected from local market in Lahore near liberty area, Pakistan.

Informed consent: All the experimental protocols were approved by the institute (UMT)

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