



Enhanced Antioxidant and Cytotoxic Potential of Nanocurcumin: Extraction, Characterization, and Comparative Bioactivity Evaluation

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Abstract

Curcumin, the major bioactive component of *Curcuma longa*, has wide-ranging pharmacological properties, such as anti-inflammatory, antioxidant, and anticancer effects. Its therapeutic potential is, however, hampered by poor aqueous solubility and poor systemic bioavailability. Nanocurcumin formulations provide a means of bypassing these constraints through enhanced solubility, absorption, and biological activity. In the present study, curcumin was isolated from *C. longa* rhizomes using Soxhlet extraction, column chromatography-purified, and characterized by thin-layer chromatography. Nanocurcumin was prepared by a top-down ultrasonication method with nanoparticles with a mean size of about 200 nm. UV–Visible spectroscopy characterization showed a significant blue shift (from 475 nm in bulk curcumin to 434 nm in nanocurcumin), indicating size-dependent optical behavior. Fourier transform infrared spectroscopy (FTIR) established structural integrity, whereas scanning electron microscopy (SEM) and X-ray diffraction (XRD) established nanoscale particle size and partial amorphization. Antioxidant activity, as defined by the Ferric Reduction Antioxidant Potential (FRAP) assay, showed enhanced radical scavenging capability of nanocurcumin compared to bulk curcumin. *In vitro* cytotoxicity in Dalton's lymphoma ascites (DLA) cells showed enhanced cell death with treatment with nanocurcumin, commensurate with enhanced bioavailability. Together, these data nominate nanocurcumin as a promising candidate for therapeutic development, meriting further *in vivo* and mechanistic studies.

Keywords: Curcumin; Nanocurcumin; Antioxidant activity; Cytotoxicity; Soxhlet extraction; Bioavailability

1. Introduction

Turmeric (*Curcuma longa*) is a perennial herbaceous plant in the family Zingiberaceae that has been valued for its utility in cooking, medicine, and cosmetics. Yellow coloration characteristic of turmeric is largely due to curcumin, a polyphenolic compound that makes up about 1–9% of turmeric's weight. Curcumin has a broad spectrum of pharmacological activities that include anti-inflammatory, antioxidant, antimicrobial, and anticancer activities. Its therapeutic value has been recognized in traditional Ayurvedic medicine and validated by contemporary biomedical research.

Molecular actions of curcumin include modulation of transcription factors, downregulation of inflammatory cytokines, and neutralization of reactive oxygen species (ROS) that underlie its protective and therapeutic actions in prevention and

treatment of chronic diseases like arthritis, neurodegenerative diseases, metabolic syndrome, and cancers[1]. Although it has potent bioactivity, curcumin is drastically limited by extremely low oral bioavailability attributed to limited aqueous solubility, high metabolism, and systemic clearance. Traditional formulations cannot achieve therapeutic concentrations in plasma, thereby restricting clinical application. Nanotechnology-based drug delivery systems provide an effective strategy to bypass these pharmacokinetic challenges[2]. Nanocurcumin, the nanosized drug of curcumin, has increased solubility, enhanced gastrointestinal uptake, and extended systemic residence. By virtue of enhanced surface area and altered physicochemical properties, nanocurcumin has the potential to enhance the antioxidant and cytotoxic activities of natural

curcumin. Earlier research has shown the therapeutic promise of curcumin nanoparticles in various disease models of inflammatory diseases, metabolic disorders, and cancers. But it is important to conduct comparative research between bulk and nanocurcumin on their antioxidant activity and cytotoxicity in an attempt to optimize therapeutic formulations.

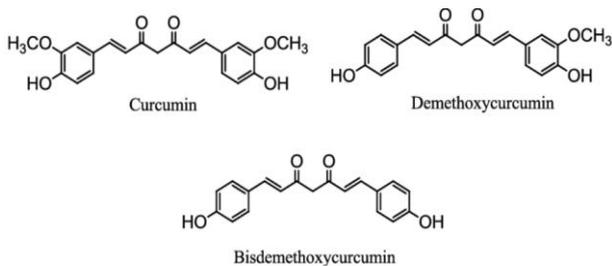


Figure 1 Molecular Structure Of Curcumin, Demethoxy Curcumin and Bis Dimethoxy Curcumin

The aim of this research was to isolate and purify curcumin from *C. longa*, synthesize and characterize nanocurcumin, and conduct a comparative analysis of their antioxidant and cytotoxic activities to unravel the enhanced biological promise with the nano formulation.

2. Materials and Methods

2.1. Extraction of Curcumin

Turmeric rhizomes were treated with a proper washing, peeling, and drying process in light-free conditions to reduce the risk of photodegradation. The dried rhizomes were milled into fine powder. Soxhlet extraction was done using ethanol as the solvent. The extract was further rotary-evaporated to obtain crude curcuminoids. Column chromatography was done for purification with silica gel as the stationary phase and a chloroform–acetone (5:1 v/v) mobile phase. Thin-layer chromatography (TLC) was done to establish the identity of curcumin, and the R_f value obtained was 0.77, which is the same as the standard value of 0.70.

2.2. Synthesis of Nanocurcumin

Nanocurcumin was prepared by a top-down ultrasonication process[3]. Curcumin (1 g) was dissolved in 20 ml dichloromethane (stock solution). During continuous ultrasonication, 1 ml stock

solution was added to boiling water (50 ml) at a flow rate of 0.1 ml/min. The solution for 30 minutes was sonicated and then centrifuged to obtain orange precipitate, which was oven-dried at 40 °C. The nanoparticles thus formed were of mean size ~200 nm.

2.3. Characterization Techniques

UV–Visible spectroscopy was performed to scan the absorption maxima (λ_{max}) of bulk and nanocurcumin in order to identify shifts characteristic of particle size diminution [4]. FTIR spectroscopy was used to characterize functional groups, ensuring structural integrity and detecting any chemical changes upon synthesis [5]. Scanning Electron Microscopy (SEM) was employed to analyze particle morphology and size distribution [6]. X-ray Diffraction (XRD) was carried out to identify crystallinity and phase transformation of curcumin and nanocurcumin [7].

2.4. Antioxidant Activity (FRAP Assay)

Antioxidant activity of curcumin and nanocurcumin was quantified using Ferric Reduction Antioxidant Potential (FRAP) assay [8]. Comparison was made using ascorbic acid standard with 25, 50, and 100 $\mu\text{g/ml}$. Absorbance was taken spectrophotometrically at 490 nm, and percentage inhibition was determined.

2.5. In Vitro Cytotoxicity Assay

Cytotoxicity was quantified by using Dalton's lymphoma ascites (DLA) cells[9]. Cell suspensions (1×10^6 cells/ml) were cultured with differing concentrations (10–200 $\mu\text{g/ml}$) of curcumin and nanocurcumin for 3 hours at 37 °C. Cell viability was assessed via trypan blue exclusion, and percentage cell death was calculated in comparison to untreated controls.

3. Results

3.1. Extraction and Purification of Curcumin

Soxhlet extraction of *Curcuma longa* yielded a yellow ethanolic extract, which suggests the enrichment of curcuminoids. Column chromatography separated the mixture of curcuminoids into colorless fractions, while the identity of curcumin was established by thin-layer chromatography (TLC) with an R_f value of 0.77, which was close to the standard value of 0.70. Purified curcumin was utilized as a raw material for

the synthesis of nanoparticles.

3.2.Synthesis and Morphological Analysis of Nanocurcumin

Top-down ultrasonication effectively synthesized nanocurcumin, and particle size was significantly reduced. Scanning electron microscopy (SEM) revealed a transition from irregularly sized microparticles, with a mean size of 3.58 μm in bulk curcumin, to spherical nanoparticles having a mean size of approximately 200 nm. The nanoparticles had a uniform morphology and decreased agglomeration, together with higher surface area-to-volume ratios.

3.3.UV–Visible Spectroscopy

UV–Visible absorption spectra revealed a blue shift of nanocurcumin compared to bulk curcumin. Bulk curcumin showed λ_{max} at 475 nm, whereas nanocurcumin showed λ_{max} at 434 nm. The shift reflects particle size decrease, as expected with higher solubility and modified optical properties. Figure 2 shows UV - Spectrum of *C. Longa* and Nanocurcumin

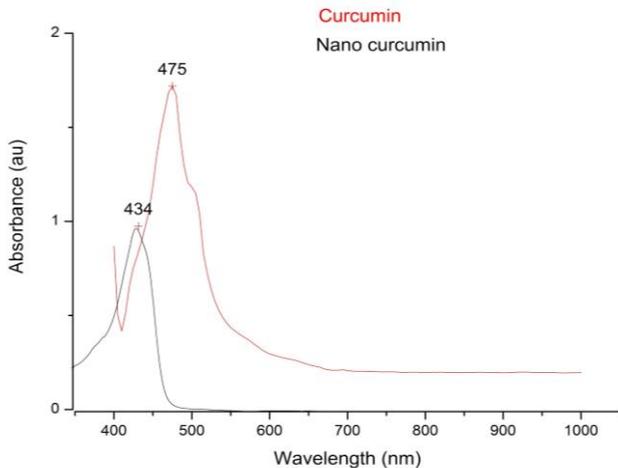


Figure 2 UV - Spectrum of *C. Longa* and Nanocurcumin

3.4.FTIR Spectroscopy

FTIR spectra of bulk and nanocurcumin exhibited characteristic peaks at 3,440 cm^{-1} (O–H stretch), 1,590 cm^{-1} (C=C stretch), 1,510 cm^{-1} (C–C aromatic stretch), and 1,279 cm^{-1} (C–O stretching). No notable new peaks were seen, which reflects that nanofabrication had not altered the chemical structure. Slight shift of the C=C stretch from 1,625

cm^{-1} to 1,590 cm^{-1} was noticed, reflecting slight molecular-level alterations due to nanosizing. Figure 3 shows IR Spectra of *Curcuma Longa* and Nano Curcumin

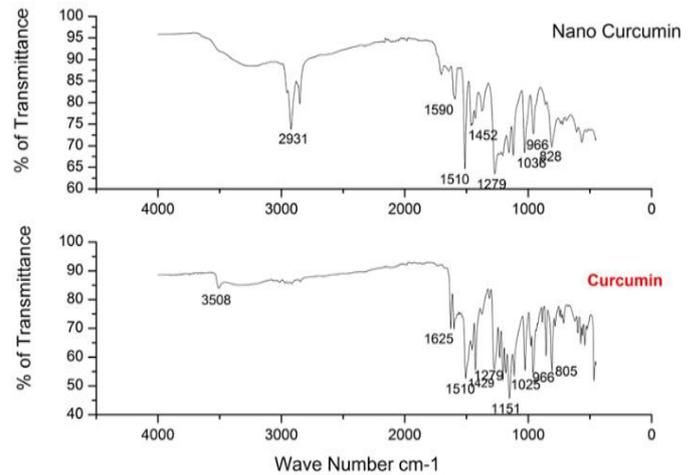


Figure 3 IR Spectra of *Curcuma Longa* and Nano Curcumin

3.5.Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscopy (SEM) micrographs of nano-curcumin are informative in terms of understanding the morphology, size, and surface properties of the particles. Nano-curcumin is the term used to describe curcumin particles that are reduced to nanoscale size, generally for improved bioavailability and therapeutic response. SEM images can provide the particle size and distribution of nano-curcumin, enabling researchers to identify the average particle size and the uniformity or dispersion in size distribution, which is essential for understanding biological properties and enhancing the synthesis process. They also give informative data regarding surface morphology, such as characteristics like roughness, porosity, and agglomeration, which are significant for forecasting interaction between nano-curcumin and biological systems. Furthermore, SEM characterization discloses the morphology of the nanoparticles, that is, spherical, rod-shaped, or irregular, that plays a critical role in cellular uptake, distribution, and clearance. Agglomeration and dispersion levels can also be evaluated, since particles may agglomerate because of electrostatic

attraction, van der Waals forces, or solvent evaporation during fabrication, eventually impacting stability and biological activity. SEM images also assist in assessing the quality of nano-curcumin samples by identifying contaminants or impurities that might change their safety and effectiveness. Figure 4(A) shows the surface morphology of the original curcumin powders, with an irregular shape and average size of around 3.58 μm , while Figure 4(B) is a display of SEM micrographs of curcumin nanoparticles showing an irregular spherical surface morphology with an average size of about 200 nm, reflecting a substantial decrease in comparison to the native powders [10]. Interestingly, spherical morph type with a very porous, sponge-like structure was found to occur in the nanoform alone, and particle size was significantly decreased from microsize to nanosize (± 200 nm), the average size of curcumin nanoparticles having been found to be 208 nm [11]. Figure 4 shows SEM Images of (A)Curcumin and (B)Nanocurcumin

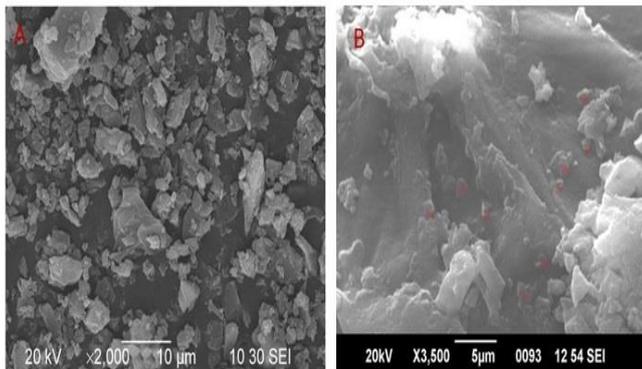


Figure 4 SEM Images of (A)Curcumin and (B)Nanocurcumin

3.6.X-ray Diffraction Analysis

X-ray diffraction (XRD) patterns showed that crystallinity decreased upon conversion to nanocurcumin. The bulk curcumin characteristic peaks at $2\theta = 13.9^\circ, 16.3^\circ, 21.1^\circ, 25.5^\circ,$ and 26.5° were weakened or lost in nanocurcumin, indicating the conversion from crystalline to amorphous structure. Amorphization is also said to increase dissolution rates, which is one of the reasons for increased bioavailability. Figure 5 shows XRD of Curcumin and Nanocurcumin

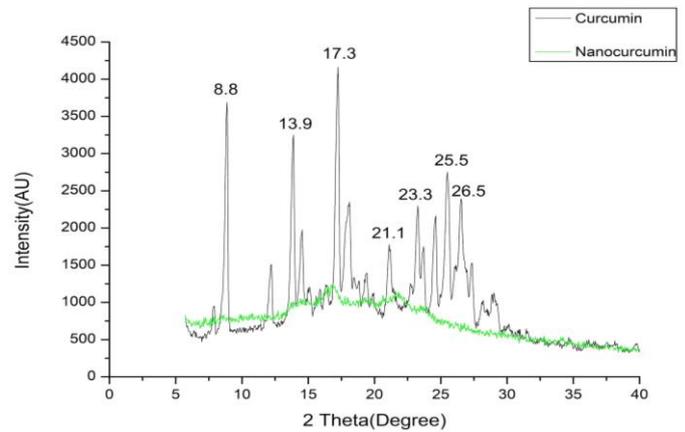


Figure 5 XRD of Curcumin and Nanocurcumin

3.7.Antioxidant Activity

Ferric Reduction Antioxidant Power (FRAP) assays showed dose-dependent radical scavenging ability by all the test samples. At 100 $\mu\text{g/ml}$, nanocurcumin inhibited 68.57%, bulk curcumin inhibited 58.69%, and ascorbic acid (control) inhibited 74.49%. The better antioxidant activity of nanocurcumin could be attributed to increased solubility and higher surface reactivity. Figure 6 shows Antioxidant Effect of Various Samples

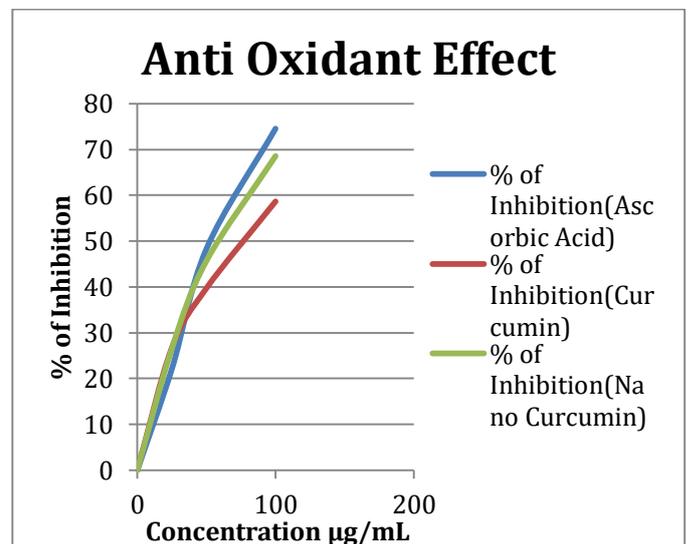


Figure 6 Antioxidant Effect of Various Samples

3.8.In Vitro Cytotoxicity

Cytotoxicity against Dalton's lymphoma ascites (DLA) cells showed that nanocurcumin and curcumin induced concentration-dependent cell killing. Nanocurcumin induced 27.1% cell killing at 200

µg/ml compared to 9.7% induced by bulk curcumin, establishing superior cytotoxic activity. This increase is in agreement with increased cellular uptake and retention of the nanosized compound. Figure 7 shows In-Vitro Cytotoxicity Against DLA Cells

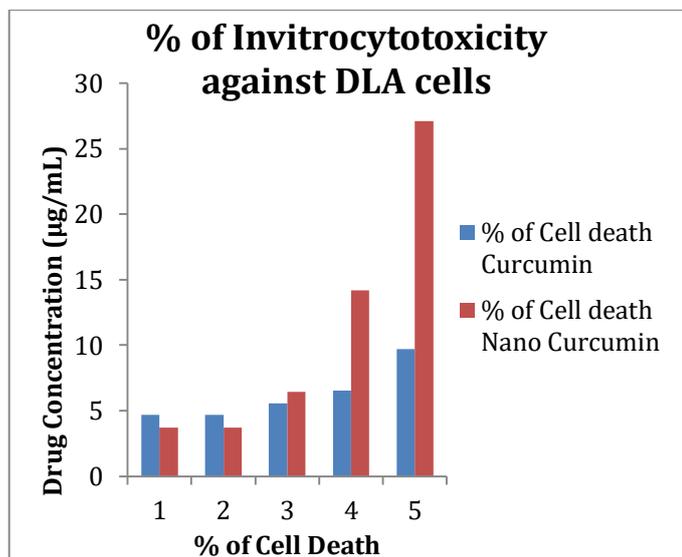


Figure 7 In-Vitro Cytotoxicity Against DLA Cells

4. Discussion

Therapeutic interest in curcumin has otherwise been limited by low solubility, instability, and low systemic bioavailability. In the present study, we demonstrated that nanosizing of curcumin significantly improves its physicochemical properties, with enhanced antioxidant and cytotoxic activity. Surface area augmentation and changed crystallinity by size reduction to ~200 nm created an amorphous form with enhanced dissolution properties, as has been observed in earlier studies with nanocrystalline drug products. The blue shift in UV–Visible spectra is a size-dependent optical response of nanoparticles. FTIR analysis proved that nanosizing is not chemically converting curcumin and retaining its bioactive functional groups. SEM micrographs showed uniform morphology, and XRD confirmed decreased crystallinity—a property that is well documented to enhance dissolution rates and, consequently, bioavailability. FRAP assay indicated that nanocurcumin was as close to ascorbic acid's level of antioxidant activity, superior to bulk curcumin at all concentrations. Given the fact that

oxidative stress is the basis of pathogenesis of most chronic diseases, the above evidence validates nanocurcumin as an even better therapeutic agent. Likewise, cytotoxicity assay indicated that nanocurcumin activity against DLA cells was almost three times higher than bulk curcumin at the maximum concentration, indicating enhanced intracellular delivery and retention.

These results align with previous reports of enhanced biological activity of curcumin nanoparticles in anticancer and antioxidant applications. Mechanistically, the nanoscale size is likely to allow for greater cellular uptake, bypass efflux transporters, and enhance systemic persistence, collectively promoting therapeutic effect.

Conclusion

This study describes nanocurcumin as a highly bioactive formulation with augmented antioxidant and cytotoxic activity compared to bulk curcumin. Soxhlet extraction and chromatographic purification yielded curcumin suitable for top-down ultrasonication to prepare approximately 200 nm average-sized nanoparticles. Characterization studies ensured chemical integrity preservation in addition to improved physicochemical properties such as amorphization and a stunning blue shift in UV absorption. Functional assays indicated augmented radical scavenging activity and cytotoxic efficacy, establishing the therapeutic promise of nanocurcumin. Future studies should focus on in vivo pharmacokinetics, delineation of mechanistic pathways with respect to cellular uptake, and formulation of optimized delivery systems for effective clinical deployment.

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