

Nanocurcumin: Preparation, Characterization and *Invitro* Antibacterial Activity against Human Pathogens

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Abstract

Curcumin, the chief bioactive component of turmeric, an active polyphenolic phytochemical, has been used since ancient times due to its high medicinal value. Besides its promising therapeutic value, the main disadvantages of curcumin are its poor aqueous solubility, less bioavailability, rapid metabolic rate and fast elimination from the body. Many studies have been carried for the past few years to enhance the properties of curcumin, eliminating their disadvantages and to maximize the use of curcumin. In the present study, a curcumin nanoparticle synthetic strategy was developed to improve the physicochemical properties and to investigate their antibacterial activity against four common human pathogens. The nanoparticles of curcumin have been synthesized by wet-milling technique and have been characterized using UV-Visible spectroscopy, FT-IR SEM, TEM and XRD. The nanoparticles have been found to have a uniform dispersion of particles with spherical shape having size in the range 10-30 nm. The newly synthesized nanoparticles were found freely dispersible in water even in the absence of any surfactants. It is also ensured that there was no change in the chemical structure of curcumin during the nanoparticle synthesis and found to be much more effective against both classes of tested pathogens namely, *E. faecalis*, *S.mutans*, *E.coli* and *P. aeruginosa*.

Keywords: Curcumin, nano curcumin, wet-milling method, antibacterial activity

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1. Introduction

Curcumin is a natural bioactive polyphenolic phytochemical isolated from the rhizome of the herb *Curcuma longa* L. (Turmeric) [1]. It has been used as a traditional herbal medicine for various diseases since ancient times. In South Asia, it is used as a spice in curries, food preservative, flavoring and coloring agent and also people used to treat the ground powder of curcumin for various ailments [2]. Curcumin inhibits cataract, kidney toxicity, stone formation, scarring and have medicinal activity against diabetes, Alzheimer's, psoriasis, arthritis, cardiovascular disease and also used for healing of wounds [3, 4].

Curcumin [(E,E)-1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione] is commonly available as a mixture along with demethoxycurcumin (~17 %), and bis-demethoxycurcumin (~6 %) among which curcumin (diferuloylmethane) (~77 %) is the major component [5]. The structure of curcumin, demethoxy curcumin and bis-demethoxy curcumin are clearly shown in **Figure 1**.

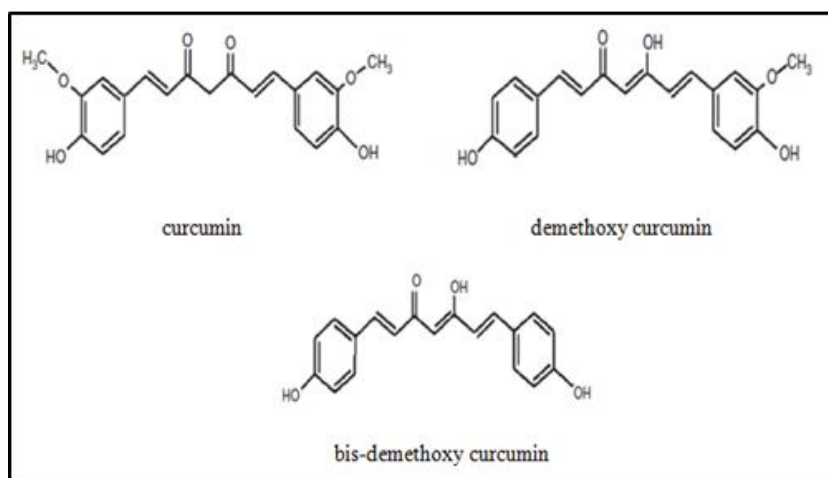


Figure 1. Structure of curcumin, demethoxy curcumin and bis-demethoxy curcumin.

Despite its nontoxic, highly potent nature, the main disadvantages of curcumin are its low aqueous solubility, poor bioavailability, fast metabolic rate and high rate of excretion [6, 7]. Enhancing the bioavailability and solubility of curcumin was one of the main concerns of researchers for the past few decades which led to the synthesis of nano formulation of curcumin to overcome the limitations. Nanotechnology is a branch of science relating the synthesis and properties of nano scale objects within the size range 1-100 nm. Nanoparticle formation increases the surface area of curcumin thereby increasing the physicochemical properties. Other common methods to improve

the limitations are binding or encapsulating curcumin with other carriers such as bio polymeric nanomaterials, inorganic nanomaterials, hydrogels, micelles, liposomes, and nano emulsions etc. which are nontoxic and biocompatible in nature [8, 9].

Various methods have been reported for the synthesis of nano curcumin, a brief outline of which is given in the schematic diagram shown in **Figure 2**. Many researchers have demonstrated the benefits of curcumin such as antimicrobial [10, 11], antiviral [12, 13], anti-inflammatory [14], anticancer [15, 16] and antioxidant activities [17]. Anand et al studied the membrane altering properties of curcumin like thinning and disruption of the membrane on artificial membranes at higher concentrations [18]. Tyagi et al studied the curcumin induced membrane permeabilization using live bacteria from both classes namely, *S.aureus*, *E.faecalis*, *E.coli* and *P.aeroginosa* and also the effect of curcumin on bacterial cell membranes at high cell densities. They checked the integrity of bacterial membrane using two differential permeabilization indicating fluorescent probes, propidium iodide and calcein and both assays confirmed the membrane leakage on both classes of bacteria by the usage of curcumin [19]. The aim of the present study was to formulate a method for the synthesis of nanoparticles of curcumin to improve its aqueous solubility and bioavailability and to examine the effect of synthesized nanoparticles on human pathogens.



Figure 2. Different methods of synthesis of curcumin nanoparticles

2. Experimental

2.1. Materials

Curcumin was purchased from the Sigma-Aldrich, USA. Dichloromethane (CH_2Cl_2) used for the preparation of nanoparticles was of analytical grade purchased from Merck, Germany.

2.2. Preparation of curcumin nanoparticles

The preparation of nanocurcumin was based on wet-milling technique [20]. 50 mg of curcumin was dissolved in 10 ml dichloromethane. This solution was added to 100 ml boiling water drop wise under ultrasonic conditions. After sonication, the solution was stirred at 200 rpm at room temperature for one hour. This was then centrifuged, washed and dried to get powdered form of nano curcumin. **Figure 3** shows the whole process of formation of curcumin nanoparticles.

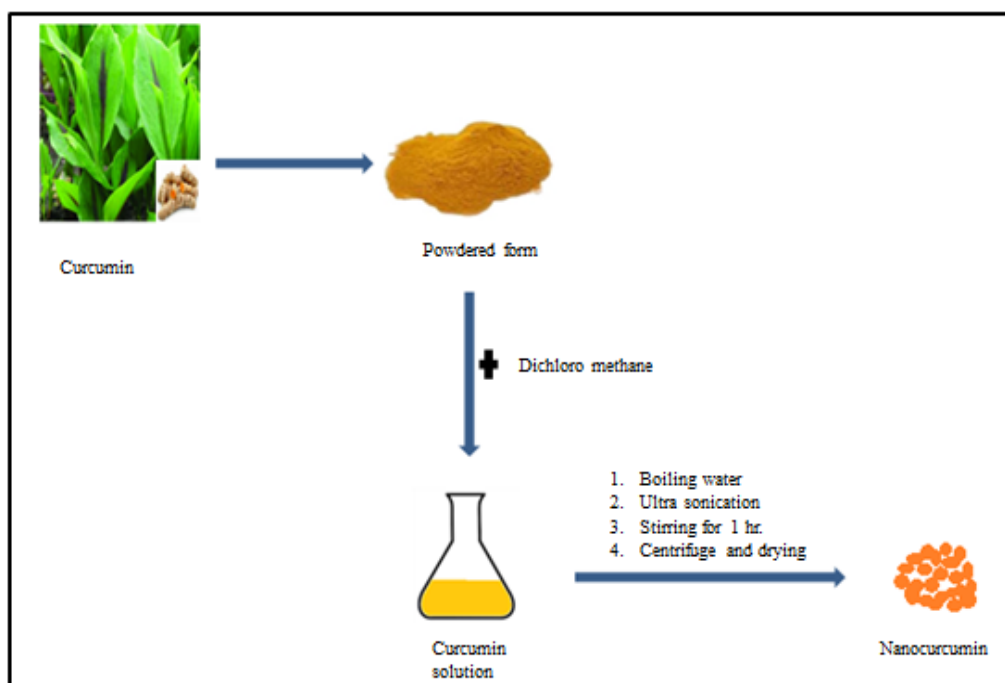


Figure 3. Schematic representation showing the synthesis process of nanocurcumin

2.3.Characterization of nanoparticles

The UV-Visible absorbance spectroscopy was carried out using Perkin Elmer UV–visible spectrometer in the wavelength range 400-800 nm. FTIR spectra were recorded by scanning the particles in the range 400-4000 cm^{-1} using Thermo Nicolet, Avatar 370' FTIR spectrometer by KBr disc method. The crystalline size and phase structure were determined by X-ray

diffractometer with high intensity CuK α radiation over the 2 θ range of 20-80 °.The average crystallite size was calculated using Scherrer equation [21].

$$t = \frac{k\lambda}{\beta \cos \theta} \quad (1)$$

where λ is the wavelength (0.15406 nm), θ is the Bragg angle, k is a constant (0.9) and 't' is the crystallite size (nm), β is the full width at half maximum (FWHM).

The morphology of the nanocurcumin was analyzed using SEM-EDS (JEOL JSM-6610-LV with Oxford EDS). Transmission electron microscopy (TEM) (JEM-1011) was used to find out the exact morphology of the nanoparticles.

2.4. Biological activity of curcumin nanoparticles

2.4.1 Determination of antibacterial activity of curcumin nanoparticles by Agar–well diffusion method

The antimicrobial studies were carried out at Biogenix Research Centre, Thiruvananthapuram, Kerala, India. The antimicrobials present in the samples were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition was measured in millimeters.

Petriplates containing 20 ml Muller Hinton Agar Medium were seeded with bacterial culture of *E.faecalis*, *S.mutans*, *E. coli* and *P. aeroginosa* (growth of culture adjusted according to McFarland Standard, 0.5 %). Wells of approximately 10 mm was bored using a well cutter and 100 μ L of sample was added. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

3. Results and Discussion

3.1. Characterization of Curcumin nanoparticles

It had already been reported that nanoformulation increases the solubility of curcumin in aqueous media [22]. The formation of nanocurcumin was indicated by the solubility of synthesized nanoparticles in water as shown in **Figure 4**.



Figure 4. Solubility of nanocurcumin in water

The UV-visible spectra in the range of 400-800 nm, of original curcumin and nanocurcumin are shown in **Figure 5**. The absorption band of curcumin was found to be at 467 nm as can be seen from the figure. But for nanocurcumin it was at 424 nm. This indicates that nano curcumin exhibits a blue shift when compared with curcumin. The size of the crystal structures influences the optical property and the absorption maxima and here the shift to lower wavelength (blue shift) is due to the reduction in size. Studies show that when size decreases, there is a shift in the absorption maxima to lower wavelengths [23]. Hence it is confirmed that the newly synthesized curcumin particles are in the nano regime which was quite in agreement with the studies reported by Rajasekar et al and Alam et al [24, 25].

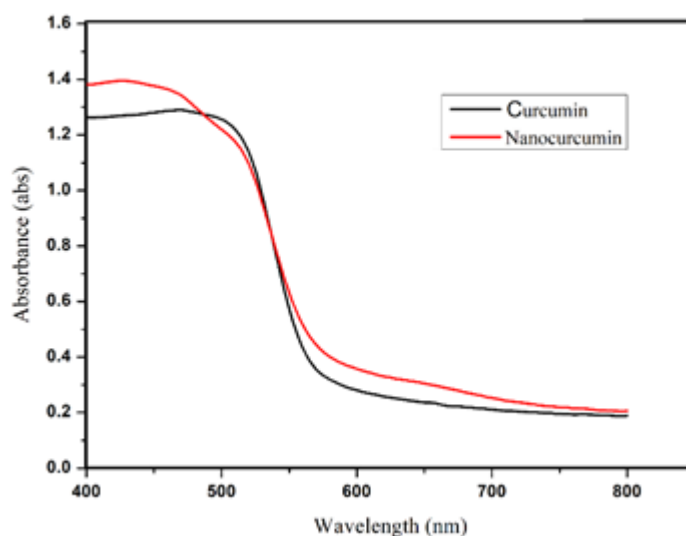


Figure 5. UV-Visible spectrum of curcumin and nanocurcumin

The FTIR spectra of curcumin and nanocurcumin are shown in **Figure 6**. The FTIR spectrum shows that the chemical structure of nanocurcumin and curcumin are the same and there was no change occurs during the nanoparticle preparation. In the FTIR spectrum peaks correspond to different functional groups. The absorption peaks of nanocurcumin showed shifts from characteristic vibrations of curcumin such as 3512 cm^{-1} (O-H), 1628 cm^{-1} (-C=O), 1509 cm^{-1} (C=H), 1155 cm^{-1} (C-H), 1026 cm^{-1} (C-N) to more intense peaks at 3509 cm^{-1} , 1623 cm^{-1} , 1512 cm^{-1} , 1157 cm^{-1} and 1029 cm^{-1} respectively [26, 27].

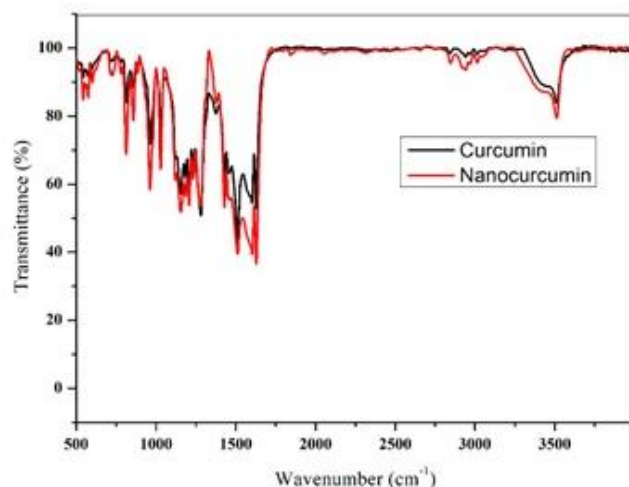


Figure 6. FTIR spectra of curcumin and nanocurcumin

The nano crystalline structure of nanocurcumin was analyzed using XRD. The XRD pattern shows main peaks at 12.09° , 14.62° and 17.29° (**Figure 7**) confirms the formation of well crystalline nanocurcumin [28]. The crystal size of curcumin nano crystals calculated using Scherrer equation was found to be 26 nm.

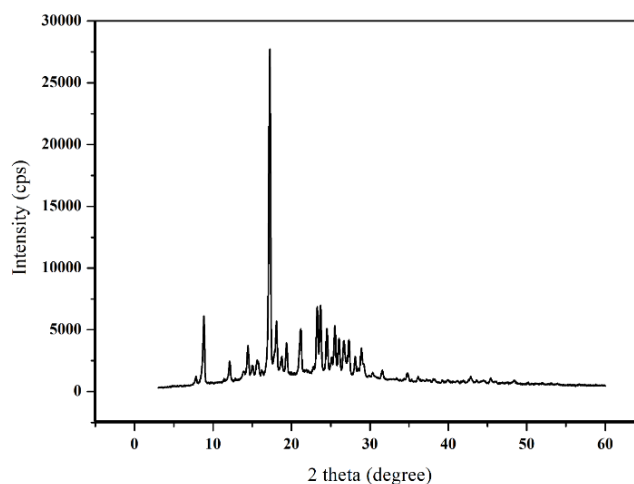


Figure 7. XRD pattern of nanocurcumin

The morphology of the synthesized nano curcumin was analyzed using SEM analysis. From the SEM images shown in **Figure 8**, it was observed that the synthesized curcumin nano crystals have spherical shape. The nano morphology of the curcumin nanoparticles was also investigated using TEM analysis. From the TEM micrographs shown in **Figure 9**, the shape of the nanoparticles was again confirmed to be spherical with size ranges from 10-30 nm. XRD data validates this observation where the size calculated using Scherrer equation is 26 nm.

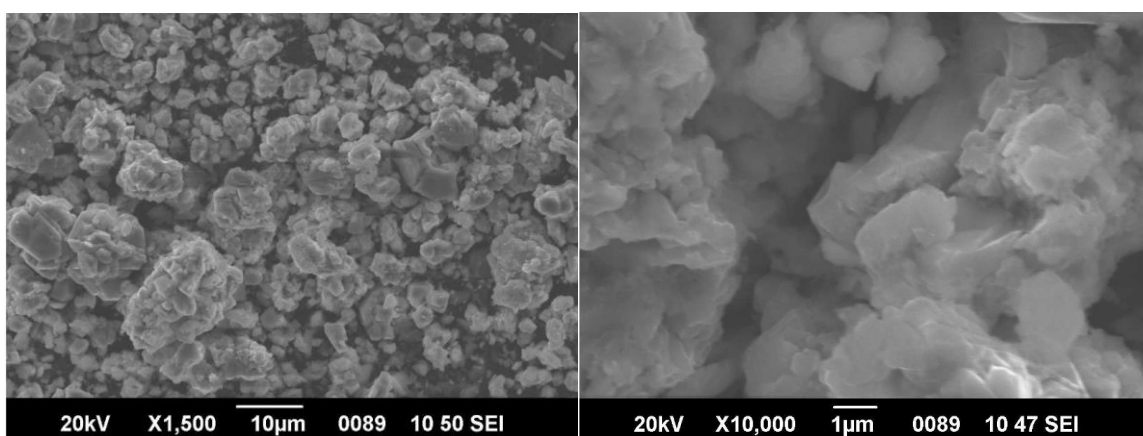


Figure 8. SEM images of curcumin nanoparticles

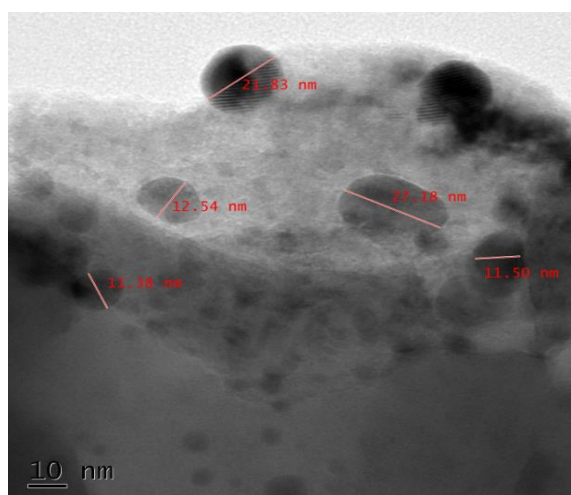


Figure 9. TEM micrograph of nanocurcumin

3.2. Antibacterial activity of curcumin nanoparticles

The newly synthesized curcumin nanoparticles were screened *in vitro* for their antibacterial activity against bacteria: *E.faecalis*, *S.mutans*, *E. coli* and *P. aeruginosa* (Fig.10). The zone

inhibition of bacterial growth was measured in mm. The antibacterial activity can be assessed based on the diameter of the inhibition zone. A larger zone of inhibition around a disk indicates that the bacteria are more sensitive to the antibiotic in the disk. The present study found that curcumin nanoparticles show effective antibacterial activity against both gram classes of bacteria. Nanocurcumin had already been reported to possess enhanced biological activity and better solubility and stability than curcumine [28-30]. The curcumin nanoparticles break the peptidoglycan layer of the bacterial cell wall and penetrate into the cell, causing a complete destruction to the structure of cell organelles which leads to cell death. It had been reported that curcumin behaves as amphipathic and lipophilic molecule, thus inserts into liposome bilayers and enhances the permeability [31]. It was also reported that curcumin disorders the 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membranes [32].

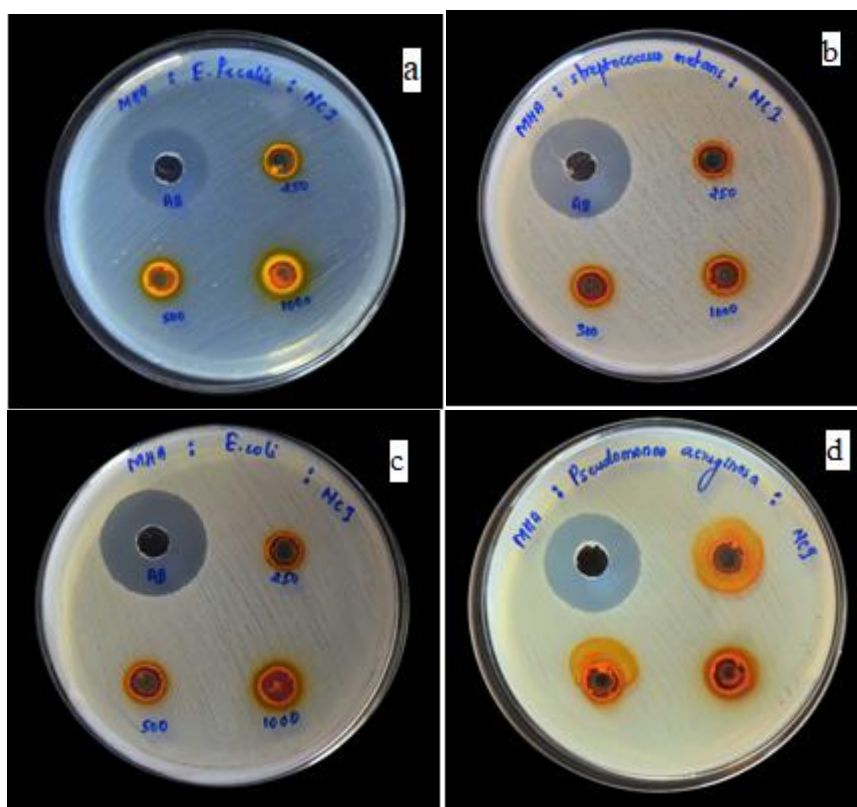


Figure 10. Antibacterial activity of curcumin nanoparticles against (a) *E. faecalis* (b) *S. mutans* (c) *E. coli* (d) *P. aeruginosa*

From **Table 1**, it is clear that nanocurcumin have the ability to inhibit all the four tested human pathogens in a concentration dependent manner i.e. the killing efficacy of curcumin increases with increase in concentration. It shows same range of inhibition against both the gram classes of bacteria.

Table 1. Zone of inhibition of nanocurcumin against human pathogens

Organism	Concentration of nano curcumin ($\mu\text{g/mL}$)	Zone of inhibition (mm)
S.mutans	250	11
	500	11
	1000	13
	Streptomycin (100 μg)	23
E.faecalis	250	10
	500	11
	1000	13
	Streptomycin (100 μg)	25
E.coli	250	11
	500	11
	1000	13
	Streptomycin (100 μg)	26
P.aeruginosa	250	12
	500	13
	1000	14
	Streptomycin (100 μg)	26

4. Conclusions

In summary, a curcumin nanoparticle synthesis system was developed and employed using wet-milling technique without the use of any surfactants. Through this method we could achieve stable narrow particle size distribution of nanocurcumin. The nanoparticles synthesized using this technique have been found to have good aqueous solubility, enhanced physical and chemical stability and can be stored as powder at room temperature. Studies on antibacterial properties of curcumin reveal that the antibacterial efficacy is enhanced upon nanoparticle formation. The antibacterial analysis confirms the inhibitory action of nanocurcumin against both gram classes of bacteria.

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