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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 solubility in water, limited bioavailability, high metabolic rate and rapid removal from the body. Many studies have been carried for the past few years to enhance the properties of curcumin, eliminating their disadvantages and maximizing 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. Wet-milling technique was employed to synthesis nanoparticles of curcumin and they were characterized using UV-Visible spectroscopy, FT-IR SEM, TEM and XRD. The nanoparticles were spherically shaped with a uniform dispersion of particles 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 the chemical structure of curcumin was well-maintained during the course of synthesis and found to be much more effective against both classes of tested pathogens namely, E. faecalis, P. aeroginosa., E.coli and S.mutans.

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

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1. Introduction

Curcumin, the key bioactive component of natural turmeric, is 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 is chemically [(E,E)-1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione] and found as a mixture of demethoxycurcumin (~17 %) and bis-demethoxycurcumin (~6 %) and curcumin (diferuloylmethane) (~77 %) is the major constituent [5]. The chemical structures of these components are shown in **Figure 1.**

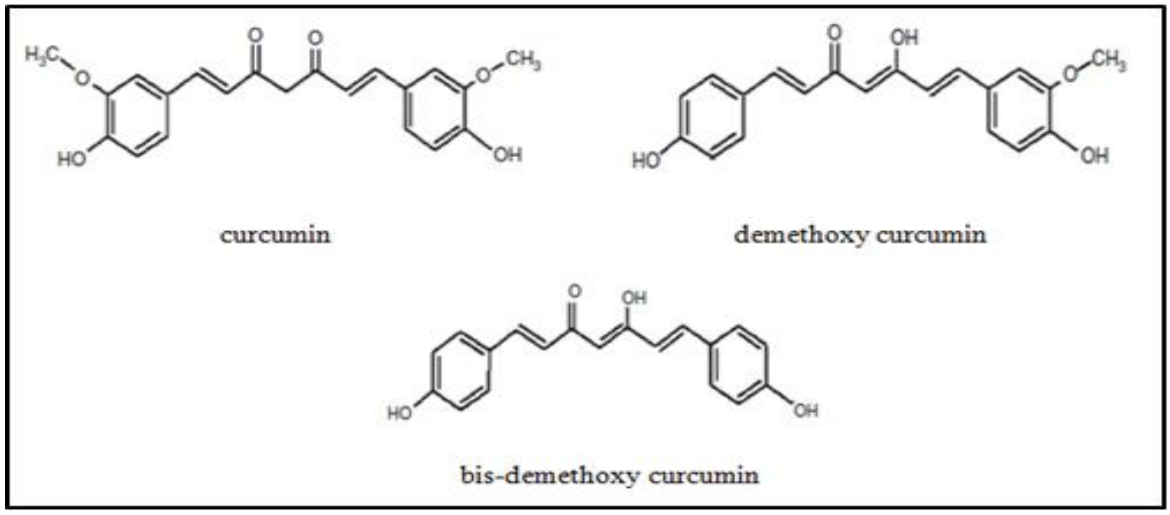

Figure 1. Structure of curcumin, demethoxy curcumin and bis-demethoxy curcumin [6].

Despite its nontoxic, highly potent nature, the main disadvantages of curcumin are its poor water solubility, less bioavailability, high metabolic rate and quick excretion [7, 8]. One of the main challenges of researchers for the past few decades was how to improve the bioavailability and solubility of curcumin which paved the way to the nano formulation of curcumin. 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 [9, 10].

Various methods have been reported for the synthesis of nano curcumin and many researchers have demonstrated the benefits of curcumin such as antimicrobial [11, 12], antiviral [13, 14], anti-inflammatory [15], anticancer [16, 17] and antioxidant activities [18]. Anand et al studied the membrane altering properties of curcumin like thinning and disruption of the membrane on artificial membranes at higher concentrations [19]. 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. Both assays confirmed the membrane leakage on both classes of bacteria by the usage of curcumin [20]. The purpose of the present study was to formulate a method for nano curcumin synthesis to increase its aqueous solubility and bioavailability and to assess the effect of synthesized nanoparticles on human pathogens.

2. Experimental

2.1. Materials

Curcumin used for the present study was procured from the Sigma-Aldrich, USA. The solvent dichloromethane (CH_2Cl_2) was obtained from Merck, Germany.

2.2. Preparation of curcumin nanoparticles

The nanocurcumin was synthesized via wet-milling technique [21]. About 30 mg of curcumin was dissolved in 15 ml dichloromethane. This solution was sonicated with boiling water for 1 h. After sonication, the solution was stirred for one hour at room temperature. The solution was then centrifuged, washed and dried to get nano curcumin powder. **Figure 2** shows the whole process of formation of curcumin nanoparticles.

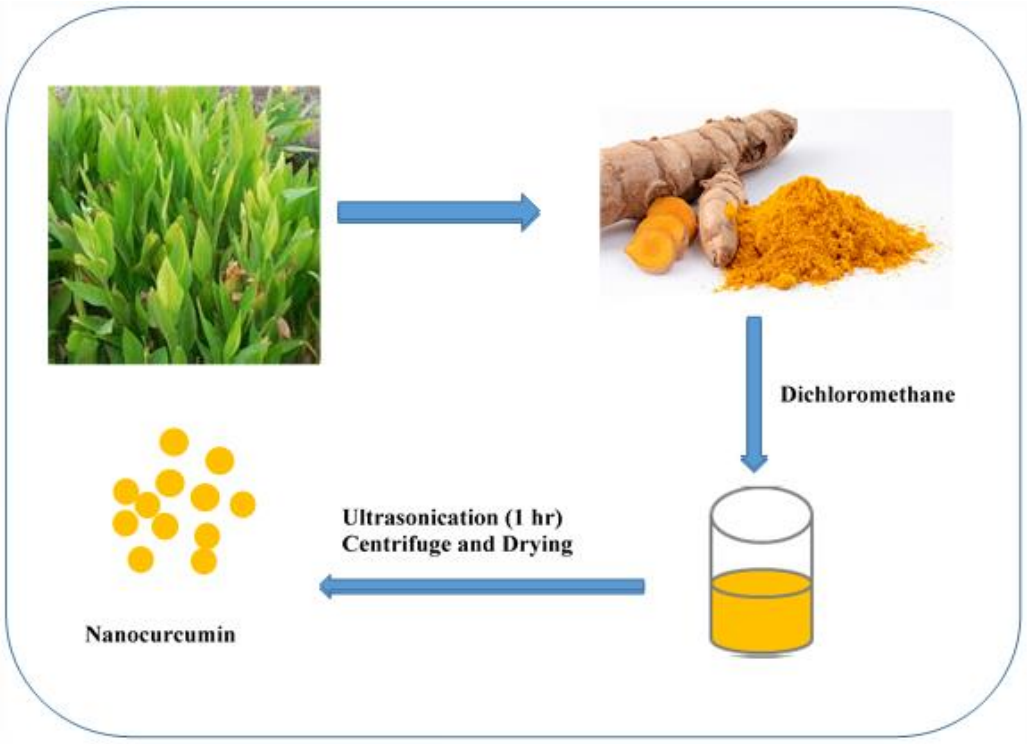

Figure 2 Synthesis of nanocurcumin

2.3.Characterization of curcumin nanoparticles

The nanoparticles were characterized using UV-Visible spectroscopy (Perkin Elmer UV–visible spectrometer in the wavelength range 400-800 nm) and FTIR spectra (Thermo Nicolet, Avatar 370' FTIR spectrometer by KBr disc method). The crystalline morphology was assessed by X-ray diffractometer with CuKα radiation over the 2θ range of 20-80 ˚. The average crystallite size was calculated using Scherrer equation [22].

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

where λ is the wavelength (0.154 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 nano morphology was analyzed using SEM-EDS (JEOL JSM-6610-LV with Oxford EDS) and transmission electron microscopy (TEM) (JEM-1011)

2.4. Biological activity of curcumin nanoparticles

2.4.1 Agar–well diffusion method

The antibacterial studies were done at Biogenix Research Centre, Thiruvananthapuram, Kerala. 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 were uniformly circular because of 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 µL of sample was added. The plates were then incubated at 37 °C for one day. The antibacterial activity was assessed by the diameter of the inhibition zone around the well (NCCLS, 1993). Streptomycin was used as a positive control.

3. Results and Discussion

3.1. Characterization of Curcumin nanoparticles

The formation of nanocurcumin was indicated by the solubility of synthesized nanoparticles in water which was in agreement with previously reported studies that nanoformulation increases the solubility of curcumin in aqueous media [23]

In the UV-visible spectra of original curcumin and nanocurcumin, an absorption peak is observed around 470 nm for curcumin and at 425 nm for nano curcumin [6]. This shows that nano curcumin exhibits a blue shift compared to curcumin which is attributed to the nanodimensuin of particles. It had already been reported that when the size decreases, there is a shift in the absorption maxima to lower wavelengths [24]. This confirmed that the newly synthesized curcumin particles are in the nano regime which agrees well with the previous reported studies [25, 26].

The FTIR spectral analysis of curcumin and nanocurcumin [6] proved that the chemical structure of nano curcumin remains the same as curcumin and no change occurs during the synthesis. In the FTIR spectrum the absorption peaks of nanocurcumin showed shifts from characteristic vibrations of curcumin such as 3510 cm^{-1} (O-H), 1630 cm^{-1} (-C=O), 1510 cm^{-1} (C=H), 1150 cm^{-1} (C-H),

1030 cm^{-1} (C-N) to more intense peaks at 3510 cm^{-1}, 1625 cm^{-1}, 15120 cm^{-1}, 1160 cm^{-1} and 1030 cm^{-1} respectively [27, 28].

The nano crystalline structure of nanocurcumin was also investigated by XRD [6] and is shown in Figure 3. The XRD pattern showed main peaks at 12.09 °, 14.62 ° and 17.29 ° which confirms the structure of crystalline nanocurcumin [29]. The calculated crystal size of nano crystals using Scherrer equation was found to be 26 nm.

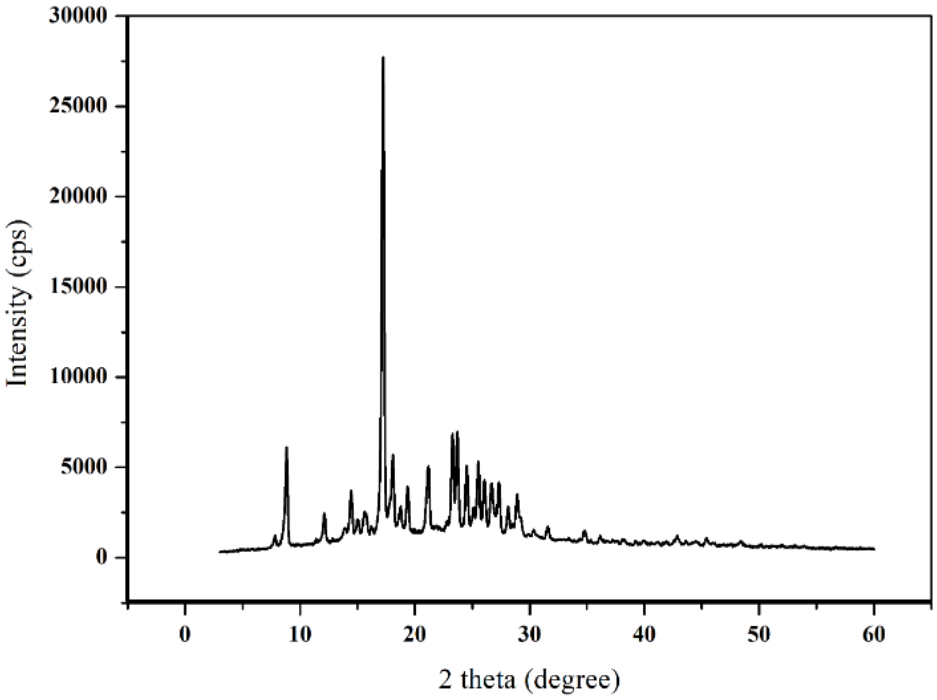

Figure 3. XRD pattern of nanocurcumin [6]

The nanomorphology of the nano curcumin was also analyzed using SEM and TEM. The SEM images shown in **Figure 4** confirms the spherical shape of curcumin nano crystals. **Figure 5** shows the TEM micrographs of the nanoparticles which supports this observation and the size ranges from 10-30 nm. This is well supported by XRD data where the particle size calculated using Scherrer equation is 26 nm.

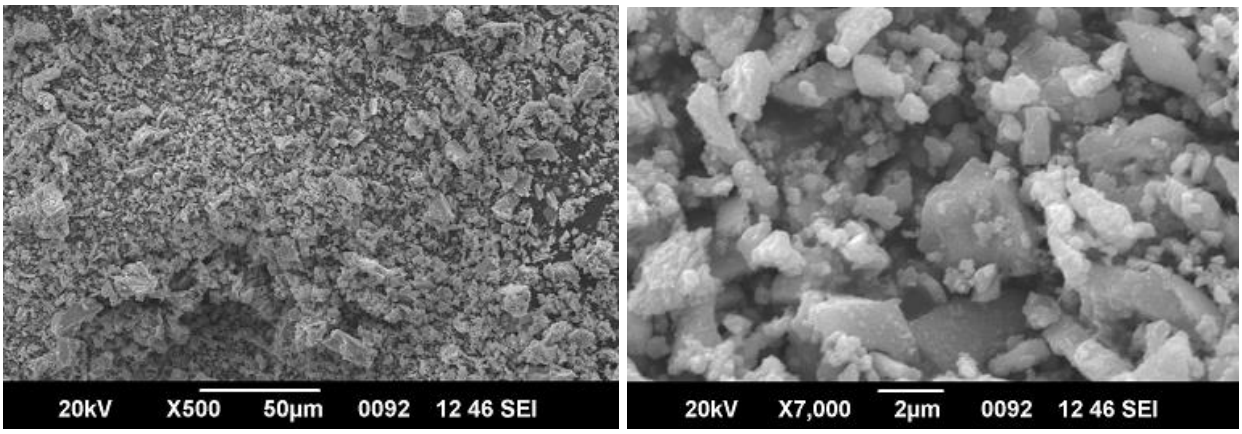

Figure 4. SEM micrographs of curcumin nanoparticles

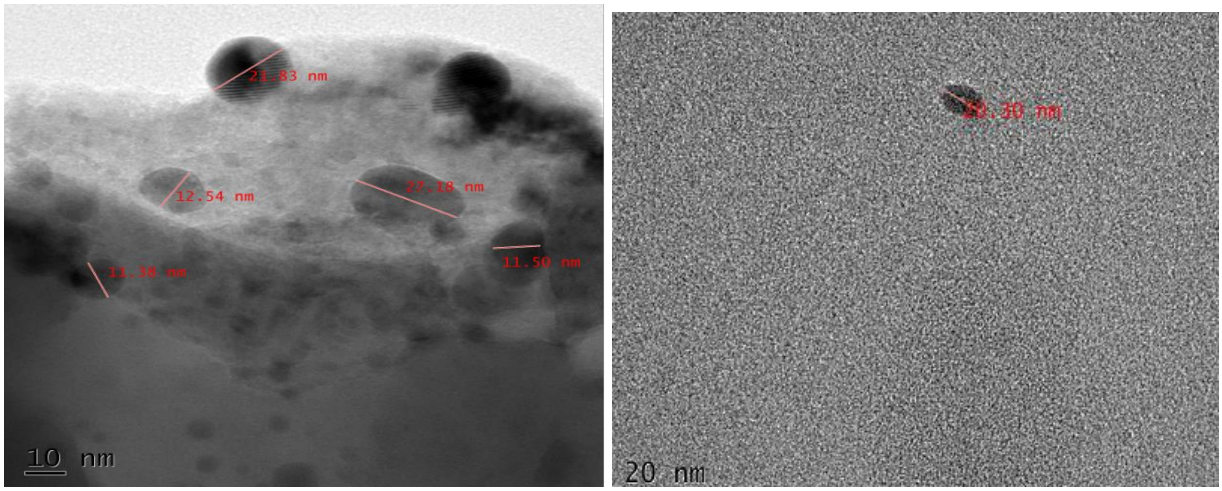

Figure 5 TEM images of nanocurcumin

3.2. Antibacterial activity of curcumin nanoparticles

The antibacterial activity of the nanoparticles was assessed for E.*coli and P. aeroginosa* (Figure 6) by measuring the zone inhibition of bacterial growth. The present study reveals that curcumin nanoparticles show effective antibacterial activity against both gram classes of bacteria. Nanocurcumin had already been reported to have enhanced biological activity and better solubility and stability than curcumin [29-32]. The curcumin nanoparticles break the bacterial cell wall and penetrate into the cell, resulting 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 [33]. Previous reports are available showing that curcumin disorders the 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membranes [34].

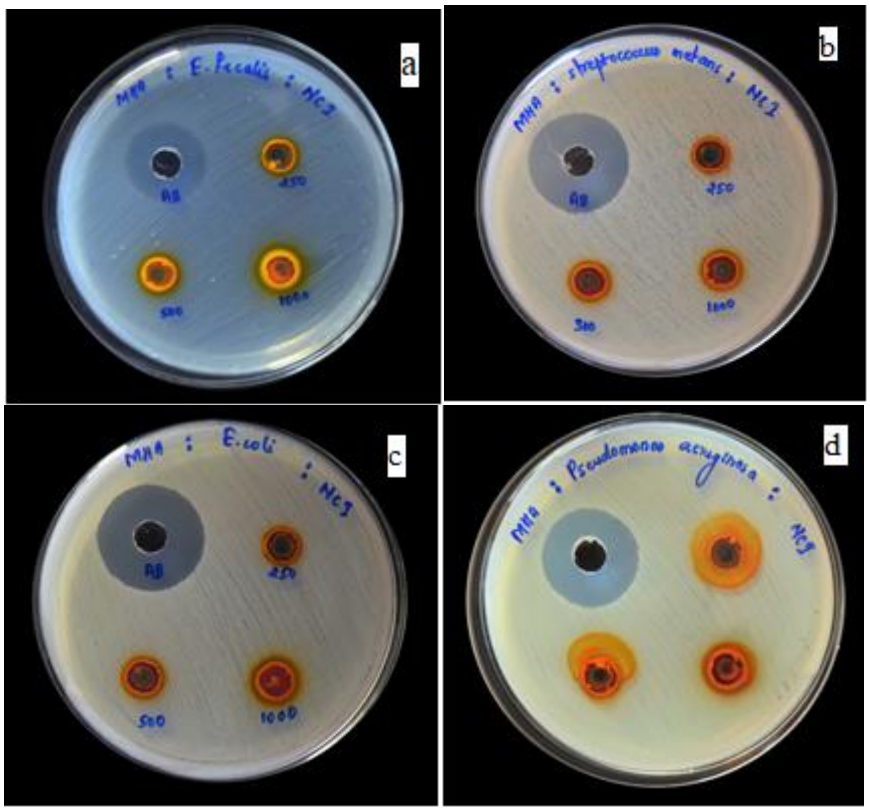

Figure 6. Antibacterial assay of curcumin nanoparticles against (a) E.faecalis (b) S.mutans (c) E.coli (d) P.aeroginosa

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 (µg/mL)	Zone of inhibition (mm)
S.mutans	250	11
	500	11
	1000	13
	Streptomycin (100µg)	23
E.faecalis	250	10
	500	11
	1000	13
	Streptomycin (100µg)	25
E.coli	250	11
	500	11
	1000	13
	Streptomycin (100µg)	26
P.aeroginosa	250	12
	500	13
	1000	14
	Streptomycin (100µg)	26

4. Conclusions

In the present work, the antibacterial efficacy of nano curcumin obtained via wet-milling technique was investigated. The nanocurcumin synthesized by this procedure has been found to possess

excellent aqueous solubility, improved stability and could be stored as a powder at room temperature. The antibacterial assay of curcumin reveals that the antibacterial efficacy is improved upon nanoparticle formation. nanocurcumin has been found to have the ability to inhibit all the four tested human pathogens in a concentration dependent manner. Thus the antibacterial studies confirm the inhibitory action of nanocurcumin against both classes of bacteria.

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