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Nanotechnology Enabled Boost In Curcumin's Antibacterial Action

[1]Padma M,[2]Vijayalakshmi M
[1]HOD Department of Microbiology,[2]Assistant Professor chemistry
[1]Department of Microbiology,[2]Department of chemistry
[1]Aurora degree & pg college, Osmania university Chikkadapally hyderabad-500044.
[2]Hyderabad institute of technology & management, JNTUH, hyderabad-501401
India

ABSTRACT : Curcumin, a yellow polyphenolic pigment from the *Curcuma longa* L. (turmeric) rhizome, has been used for centuries for culinary and food colouring purposes, and as an ingredient for various medicinal preparations, widely used in Ayurveda and Chinese medicine. In recent decades, their biological activities have been extensively studied. Curcumin is the principal curcuminoid of the popular Indian spice Curcuma longa (Turmeric), which is a member of ginger family (Zingiberaceae). Inspite of showing extraordinary medicinal properties its commercialized formulation is still a challenge because of its poor solubility, bioavailability and rapid plasma clearance. In our study, different shapes of nanocurcumin are prepared in alcohol-water solutions using sonication method. The nanocurcumin in the suspension have been characterized by UV-spectrometry. Curcumin was extracted using the Soxhlet extraction, Kirby Bauer methods. The in vitro antimicrobial activity (Inhibition) of the nanocurcumin has been compared with that of the normal curcumin using Broth dilution and Kirby-Bauer methods against both Gram positive and Gram-negative bacteria. The efficacy of nanocurcumin is better than curcumin. It was observed that nanocurcumin had better dispersibility and enhanced bioavailability in hydrophilic environment as compared to normal curcumin.

KEYWORDS: Nanocurcumin, curcuminoids, malonyl-CoA, anti-inflammatory, anticancer, antidiabetic, antioxidant, Nano formulations, TEM, Depolarization ratio, Inhibition, Soxhlet extraction, Kirby Bauer Method, Sonication, Spectrophotometer.

INTRODUCTION

The Curcuma genus has a long history of medicinal applications, being composed of approximately 120 species. Curcuma longa L. (Curcuma; Turmeric) is the most widely cultivated plant in many regions of the world. Rhizomes are the most commonly used plant part composed of a wide variety of compounds, including the bioactive non-volatile curcuminoids (curcumin, dimethoxy-,and bisdemethoxycurcumin) and the compounds present in volatile oil (mono and sesquiterpenoids) A number of beneficial pharmacological properties have been attributed to the Curcuma species, including antiproliferative, anti-inflammatory, anticancer, antidiabetic, hypo cholesterolemic, anti-thrombotic, antihepatotoxic, anti-diarrheal, carminative, diuretic, antirheumatic, hypotensive, antimicrobial, antiviral, antioxidant, larvicidal, insecticidal, antivenomous, and anti-tyrosinase effects, among others. The historic background of the Curcuma species dates back to 5,000 (Ayurveda) and 2,000 (Atharveda) years ago, respectively. C. longa contains different curcuminoids.

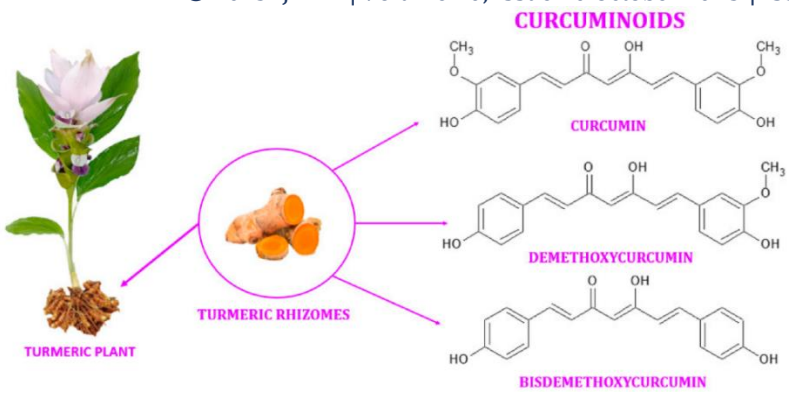

figure. purification of curcumin from curcuminoids, desmethoxycurcumin and bis desmethoxycurcumin

Curcumin incorporates (Figure.A&C) a seven-carbon linker and three major functional groups: an α, β-unsaturated β-diketone moiety and an aromatic O-methoxy-phenolic group. The aromatic ring systems, which are phenols, are connected by two α, β-unsaturated carbonyl groups. It is a diketone tautomer, existing in enolic form in organic solvents and in keto form in water. The diketones (Figure.B) form stable enols and are readily deprotonated to form enolates; the α, β-unsaturated carbonyl group is a good Michael acceptor and undergoes nucleophilic addition. Because of its hydrophobic nature, curcumin is poorly soluble in water but is easily soluble in organic solvents

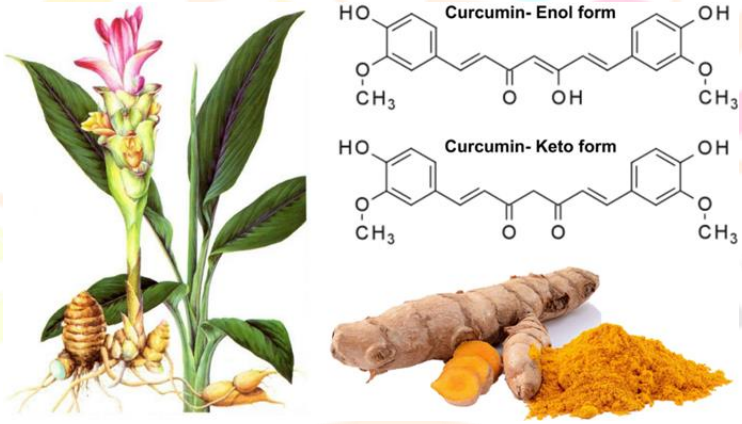

Figure.B curcumin enol-keto form

In 1973, Peter J. Roughley and Donald A. Whiting proposed two mechanisms for curcumin biosynthesis. The first mechanism involves a chain extension reaction by cinnamic acid and 5 malonyl-CoA molecules that eventually arylize into a curcuminoid. The second mechanism involves two cinnamate units coupled together by malonyl-CoA. Both use cinnamic acid as their starting point, which is derived from the amino acid phenylalanine.Plant biosynthesis starting with cinnamic acid is rare compared to the more common p-coumaric acid. Only a few identified compounds, such as anigorufone and pinosylvin, build from cinnamic acid.

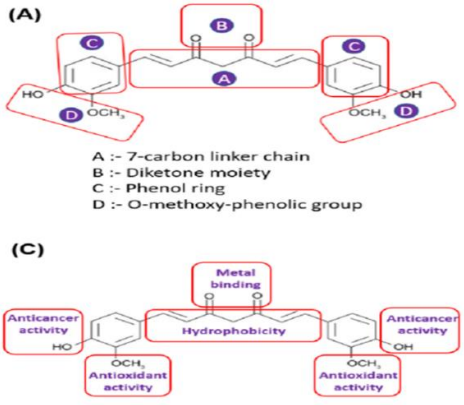

Figure.A&C. The aromatic ring structure of curcumin

This scenario originated the concept to revive the interest in natural antibacterial products of plant origin. Natural antioxidant-based antibacterial products have diverse, complex chemical structures that cannot be copied by microbes to create resistance. Antioxidants, such as polyphenols, are organic compounds mainly extracted from natural sources with antibacterial activity dominantly involved in improving the immunity of humans against various pathogens. With the development of nanotechnology, Nano-sized antibacterial compounds have attracted greater attention due to improved antimicrobial activity compared to that of bulk compounds.

Curcumin initiates multiple mechanisms of cell death in microorganisms (Figure.E). The antimicrobial activity of curcumin is incompletely characterized, but by interacting with numerous molecular targets and transduction pathways, it employs a multi mechanistic anti-infective strategy. Because of poor solubility in an aqueous phase, curcumin is categorized as a BCS (Biopharmaceutical Classification System) IV drug. The antibacterial activity of curcumin is weakened due to high lipophilicity and low cell permeability. Nano formulations of curcumin improve solubility, bioavailability, transmembrane permeability, prolonged plasma half-life, long-term stability, target-specific delivery, antimicrobial activity, and upgraded therapeutic effects. Concerning antimicrobial activity, nanocurcumin has been reported to be more effective against Gram-positive bacteria than Gram-negative bacteria

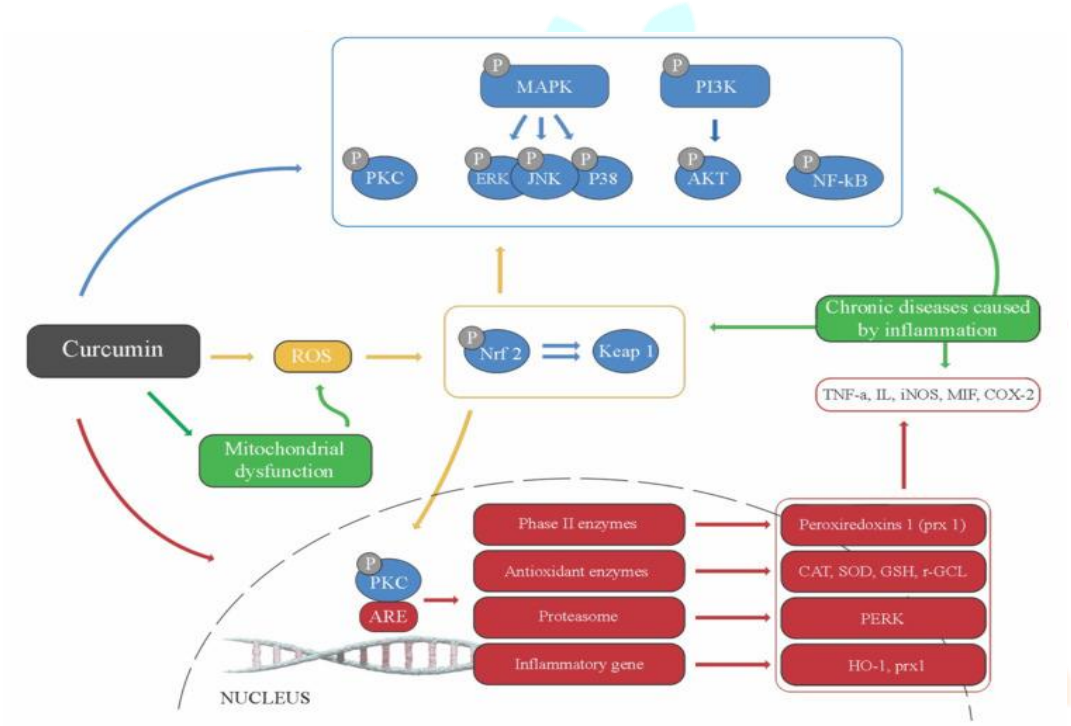

Figure.D. curcumin anti-inflammatory mechanisms

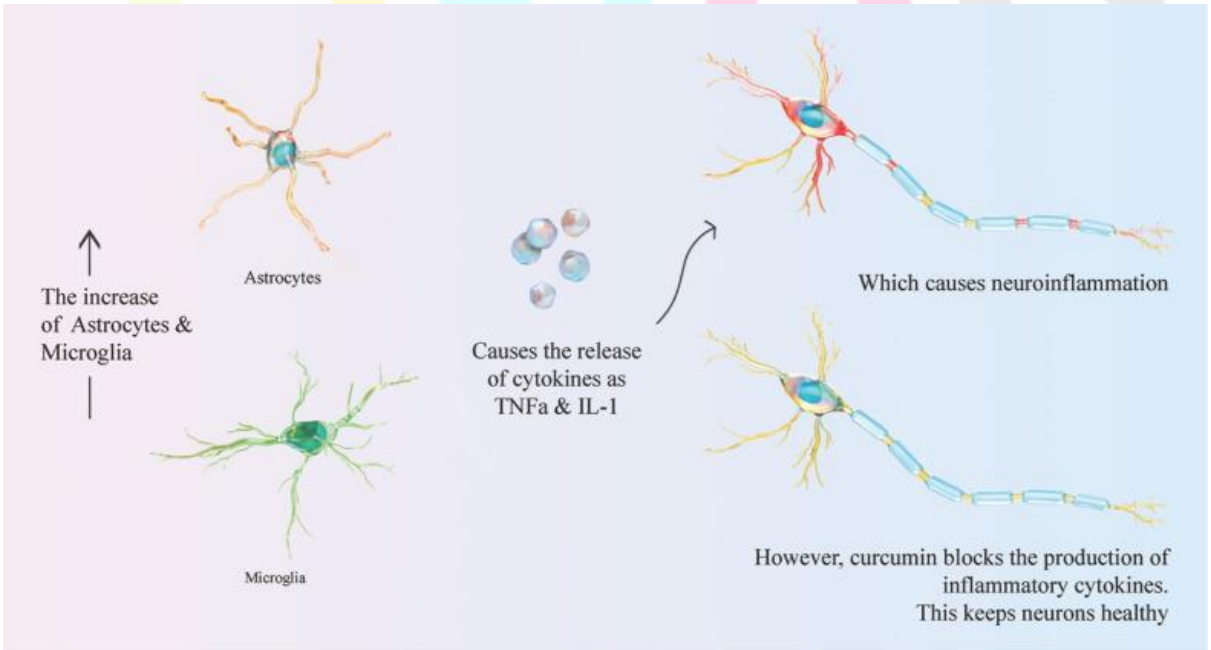

Figure.E Curcumin mechanism of action in neuroinflammation.

MATERIALS & METHODS

Extraction of Curcumin

Rhizomes were cleaned well; the rhizomes were separated and blanched in a closed pot filled with 3/4 of water for 30 min and 45 min, respectively. The rhizomes were dried under sunlight until the moisture percentage was reduced to 10%. Then, the removal of the skin in the rhizomes was done by hand, and it was ground to powder using the grinder. The moisture percentage of the turmeric powder was determined by the Dean and Stark method which was used to determine the volatile oil percentage of turmeric powder. The Soxhlet extraction method was used to determine the oleoresin percentage using ethanol as the solvent.

Curcumin was extracted using the Soxhlet extraction method. To do so, ground turmeric powder was weighed. A sample of 15.00 g was embedded in a thimble and put in the soxhlet apparatus, which was gradually filled with ethanol as the extraction solvent. The extraction was carried out at 60°Cfor 8 h by then; all the colored compounds are extracted to the solvent. Upon completing the extraction, the ethanol was separated from the extract using a RB flask heater. 1.00 g of the crude extract was mixed with 25.0 mL of hexane, stirred gently, and was kept for 12 hr. The solution was then stirred using the magnetic stirrer at 600 rpm for 3 h to obtain the powder. The solution was centrifuged. The powder obtained was separated and dried at 40°C in the oven for 2 h. 1.00 g of the crude curcuminoid powder was mixed with 10.0 mL of a hot solvent mixture of isopropyl alcohol: hexane in 1:1.5 molar ratio. The solution was then cooled at room temperature to obtain pure crystalline curcumin, and the extracted curcumin was separated by filtration

All experiments were carried out at room temperature (25°C). The bacterial strains used were E. coli DH5α and gram Negative – Cocci. All Chemicals used in the biological studies were bought from Hi-Media unless and otherwise mentioned.

PREPARATION OF NANOCURCUMIN

Nanocurcumin was synthesized by a physicochemical fabrication method. The stock of curcumin solution (5.00mg/mL) was prepared by dissolving the extracted curcumin powder in 20.0 mL of dichloromethane. 1.00 mL of stock solution was added to 50.0 mL of boiling water, in a drop wise manner at 0.1 mL/min flow rate, under ultra-sonication conditions. The solution was sonicated for 30 min. Then, the mixture was stirred at 800rpm for 20 min till an orange-colored precipitate was obtained. The supernatant was discarded, and the synthesized nano-curcumin was obtained for further studies. The various combinations of parameters like water concentration in the solvent medium (10% to 70%), sonication time (5 min and 10 min) and initial curcumin concentration (0.05% and 0.5%) were used in different trials to get an optimum combination of these parameters at which more stable curcumin nanoparticles were forming in the desired size range.

In vitro antimicrobial activity of curcumin and nanocurcumin the antimicrobial activity of curcumin and nanocurcumin against both gram positive & negative strains were studied and compared in vitro using broth dilution and Kirby Bauer methods.

Preparation of Cultures The bacterial strain was transferred from stored plates at 4°C to 10ml of nutrient broth (NB), and cultivated overnight at 37°C. The freshly grown overnight bacterial cultures were then diluted in sterile 0.8% saline solution and adjusted to a cell suspension of 5×105 colony forming units (cfu)/ml (0.5 McFarland) using UV spectrophotometer at 600 nm.

Broth Dilution Method Stock solutions of curcumin and nanocurcumin were prepared in 70% ethanol. Using this stock solution, various dilutions of curcumin and nanocurcumin samples were prepared in nutrient broth to get final concentrations of 100 µg/ml, 200 µg/ml and 300 µg/ml. The media was then inoculated with 100 µl of the freshly prepared bacterial suspension. The inoculated tubes are incubated at 37°C for 24 hours. After incubation the cultures were serial diluted to attain 10-7 dilution. 1 ml of the diluted culture was then plated on nutrient agar plates (in duplicate) and incubated at 37°C for 24 hours. A negative (untreated) and positive (ethanol treated) control were included in all the trials and all the steps were carried out in aseptic conditions. After incubation the number of colonies grown in each plate was counted.

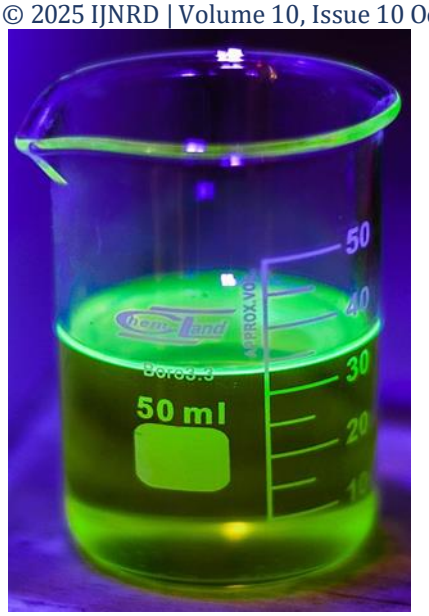

Figure.F. curcumin displays green fluorescence under uv light

The percentage growth inhibition was then calculated using the following formula:

% Growth Inhibition = (1-T/C) × 100 (10)

Where, T = cfu/ml of the test sample

C = cfu/ml of the control

Kirby Bauer Method The sample loaded discs (dia 6mm) were then carefully placed on nutrient agar plates inoculated with the test organisms. The plates were then incubated at 37°C for 24 hours. After incubation the zone of inhibition around the discs was observed and the results were photographed. All antimicrobial studies were carried out in triplicates and the averages of the results were used for final calculations.

Study of effect of curcumin and nanocurcumin on bacterial DNA the DNA of the treated bacterial samples (Control, curcumin and nanocurcumin treated) were isolated using standard protocol i.e. by salt precipitation method. In brief the DNA from the cultured bacterial cells was isolated by using Glucose-Tris EDTA mix, 1% SDS & 10mM NaCl. The DNA was precipitated by using isopropanol and the isolated DNA was subjected to agarose gel electrophoresis. The DNA bands obtained in the agarose gel were photographed using doc.

Preparation and characterization of nanoparticales the stable curcumin nanoparticles were prepared in ethanol/water solutions by the method of sonication assisted solvent worsening. The difference in solubility of curcumin and nanocurcumin in 70% ethanol and curcumin in water was shown.

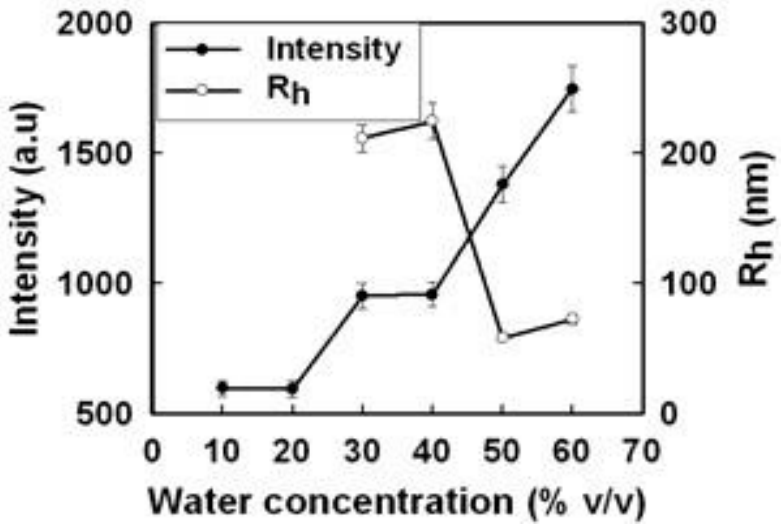

Figure G: Showing change in Intensity and R_h with increase in water ratio in the solvent. Solid lines are guide to eye.

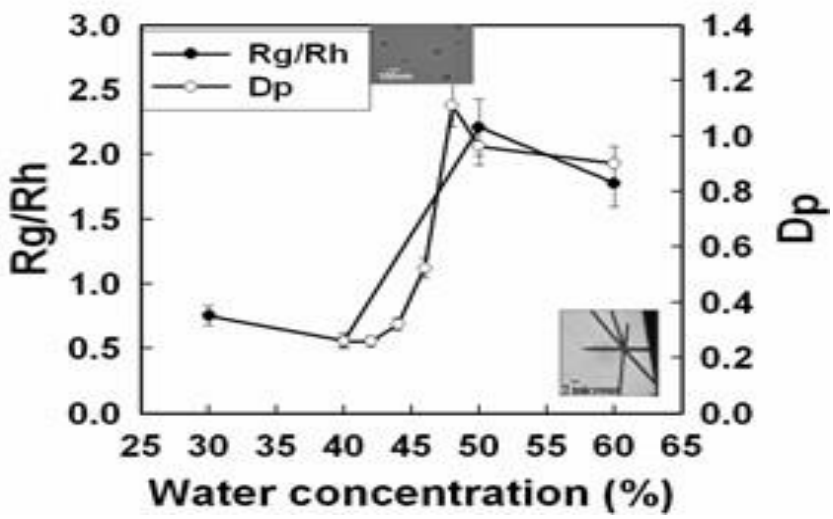

Figure H:Showing isotropic to anisotropic shift in shape of Nanocparticles with increase in water concentration in the solvent medium

(Fig G and H) shows the changes taking place in intensity, R_h and shape of nanoparticles with increase in water concentration in the curcumin solution (in ethanol) of initial curcumin concentration 0.05% (constant water addition rate accompanied by 5min sonication). The change in shape with increase in water/alcohol ratio is the most interesting result observed in this study. To understand and follow the series of changes taking place in the shape of nanoparticles SLS and TEM studies were done. The TEM images (Figure. I) supported the SLS data and visually illustrated the isotropic to anisotropic transition in the shape of curcumin nanoparticles with increase in water concentration in the solvent medium.

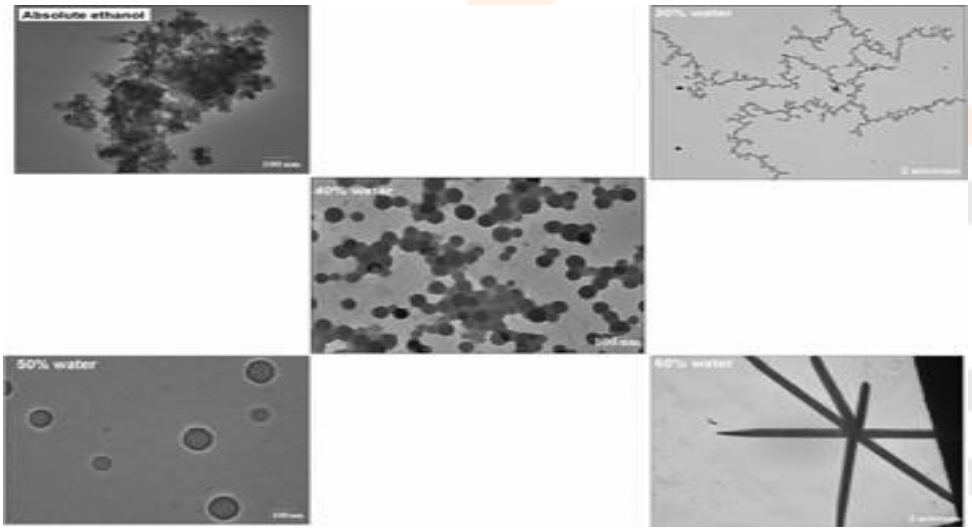

Figure J. Showing TEM images of curcumin nanoparticles in solutions of different water to ethanol ratio

The bacterial growth inhibition capacity of curcumin and nanocurcumin was calculated from broth dilution method and the comparative results are shown in (Fig J). The calculated IC_{50} of curcumin on gram negative bacteria *E. coli* was 653.75 ȝg/ml, and lower for gram positive *Cocci* (513.47ȝg/ml). But nanocurcumin showed IC_{50} of 728.48 ȝg/ml on gram negative bacteria *E. coli* and for gram-positive *cocci* it was observed to be 542.79 ȝg/ml.

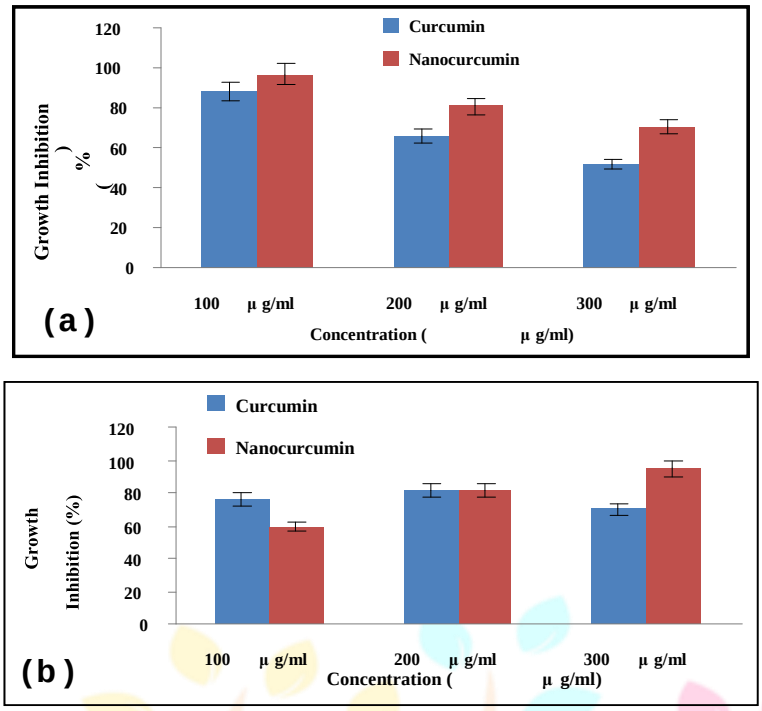

Figure K:Showing the comparison on antimicrobial activity of curcumin and nanocurcumin against (a)Gram-positive and (b) Gram-negative (E. coli) organisms

(a) Curcumin and (b) Nanocurcumin treated discs against gram-positive bacteria

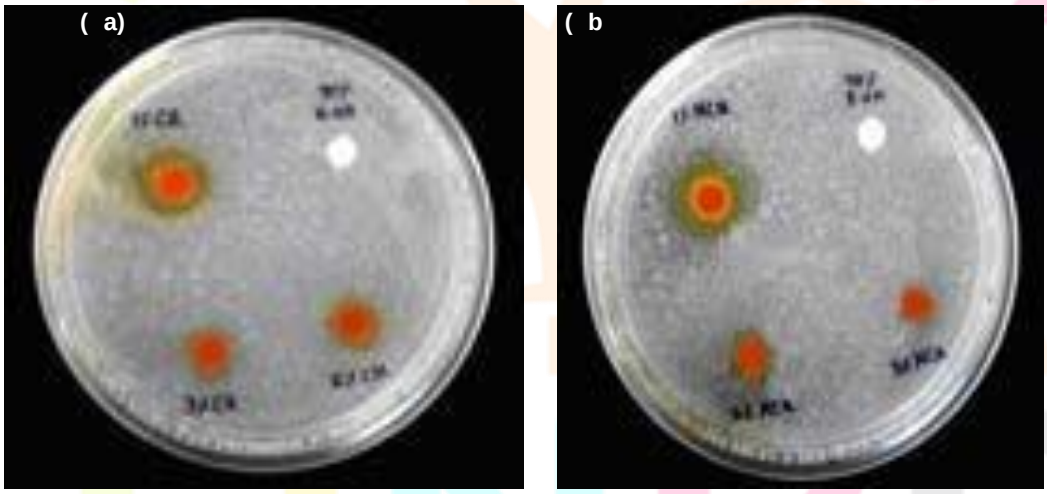

Figure L: Showing zone of inhibition in agar disc assay
(a) Curcumin and (b) Nanocurcumin treated discs against gram-negative bacteria

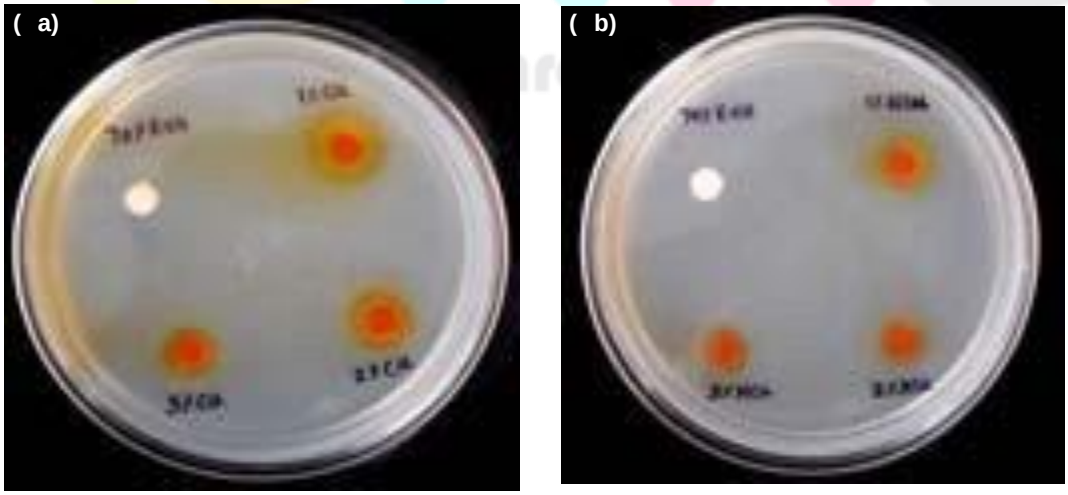

Figure M: Showing zone of inhibition around

Time dependent variation in the zone of inhibition was observed by the Agar disc assay. After 10 hrs there was a clear inhibition zone which became unclear after 24 hrs in both the curcumin and nanocurcumin treated discs. This showed that the samples are not bactericidal but only bacteriostatic to the tested strains of bacteria. (Figure.L & M). The effect of various doses curcumin and nanocurcumin were used to assess the genomic DNA damage of bacteria was investigated using agarose gel electrophoresis (Figure.N). No degradation of DNA was observed. The genomic DNA appears to be intact in all the samples.

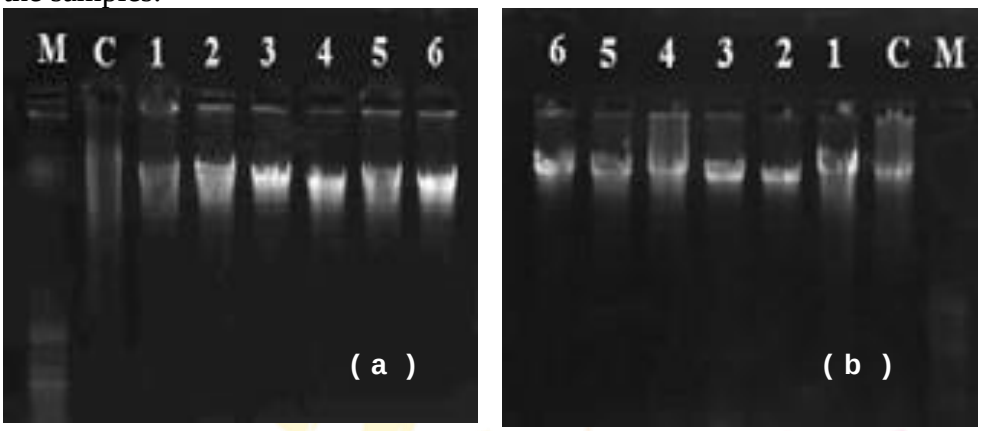

Figure N: Showing the result of agarose gel electrophoresis of DNA of (a) Gram – positive and (b) Gram – negative bacteria treated with different concentrations of curcumin and nanocurcumin
M-Marker, C-Control,1, 2 and 3 - DNA of bacteria treated with 100 µg/ml, 200 µg/ml and 300 µg/ml of curcumin respectively, 4, 5 and 6 - DNA of bacteria treated with 100 µg/ml, 200 µg/ml and 300 µg/ml of nanocurcumin respectively

DISCUSSION

The DLS study (Figure.G) revealed that the increase in water concentration in 0.05% curcumin ethanol solution at 5min sonication causes a gradual increase in intensity. The first sign of nanoparticles in the solution was observed at 30% of water concentration in the solvent, revealing that crystal nucleation started when the concentration of water in the solvent medium is ~ 20%- 30%. Further addition of water, gradually increases the formation of nanocurcumin in the solution, and the intensity rapidly and linearly increased after water concentration of 40%. It was observed that a 0.05% concentration of curcumin solution (in ethanol) can withstand solvent worsening until the water/ethanol ratio reaches 6/4. Beyond which particles precipitated on overnight standing, showing the instability of curcumin in more polar conditions. The hydrodynamic radius R_h of nanoparticles was so large (~ 220 nm) in the beginning of nanoparticle formation, where as in the later stages reduction in R_h was observed and it was around 90 nm. This reduction in particle size may be due to the effect of prolonged sonication carried out during the solvent worsening process. The SLS study showed the changes are taking place in shape of nanoparticles with increase in water/ethanol ratio. The changes in R_g/R_h and D_p (Depolarization ratio) values, the two important factors directly dependent on the shape, revealed that there is a gradual transition from isotropic anisotropic state in the shape of nanoparticles.

The phase diagram (Figure. H) showed the gradual shape transition from sphere (isotropic state, Rg/Rh = 0.754, Dp = 0.259) to rods (anisotropic state, Rg/Rh = 1.77, Dp = 0.9) with increase in water concentration.

The TEM images (Figure. I) supported the SLS data and visually demonstrated the series of changes taking place during the solvent worsening process. The normal curcumin in the absolute ethanol looks like a huge cluster (around 500 nm) and as the process of solvent worsening proceeds the cluster breaks to form individual nanoparticles of size range 100-160 nm. The water addition initiates the particle formation, whereas the sonication breaks the particles and restricts its size to nonorange. The maximum inhibition recorded at the minimum concentration of 100ʒg/ml was 88% and 97% for curcumin and nanocurcumin respectively. The trend looks completely different against Gram-negative bacteria, in which the activity of Curcumin was concentration independent in normal form whereas in nanoform it becomes concentration dependent showing a linear increase in activity with increase in concentration. The maximum percent inhibition of Gram-negative bacteria by curcumin was 81 and 95% in normal (200ʒg/ml) and nanoforms (300ʒg/ml) respectively.

From agar disc assay it was evident that both curcumin and nanocurcumin exhibited strong bacteriostatic activity. The size of nanocurcumin was around 100– 160 nm (from TEM), which is much less than the size of normal curcumin (500–800 nm), which enhances penetration and higher uptake by the cells. It is known that Gram-positive bacteria contain an outer peptidoglycan layer, while Gram-

negative bacteria contain an outer phospholipidic membrane, both of which undergo different types of interaction when encountered by curcumin.

The agarose gel electrophoresis showed that the bands of DNA isolated from control bacteria and samples (curcumin and nanocurcumin) treated bacteria appears very clear and similar to each other. Thus, the results revealed that both curcumin and nanocurcumin were not showing any significant effect on the DNA of bacteria. Hence further studies are required to understand the effects of curcumin and nanocurcumin on bacteria at the molecular level.

CONCLUSION

Nano formulations have proved to be advantageous to balance the solubility and bioavailability of various hydrophobic drugs. In case of curcumin also, Nano formulations showed promising results to overcome its limitations. A method of sonication assisted solvent worsening for the preparation of polarity withstanding nanocurcumin had been optimized in this study. It had been found that the particles were more stable when prepared in solution of ethanol/water ratio 1; with 0.05% initial curcumin concentration using 5min sonication. A gradual shape transition from isotropic to anisotropic state with increase in water concentration had also been observed. We also report the preparation of different shapes of nanocurcumin with enhanced biological activities which compared to curcumin *per se*, but optimizing the Nano formulation to make it stable in water for its therapeutic acceptability is the immediate concern.

We also conclude that nanocurcumin exhibited better *in vitro* antimicrobial activity than curcumin *per se*. Neither curcumin nor nanocurcumin was showing any significant effect on the genomes of bacteria. Hence, further studies are warranted to understand the molecular level effects of curcumin and its nanoform on bacterial cells.

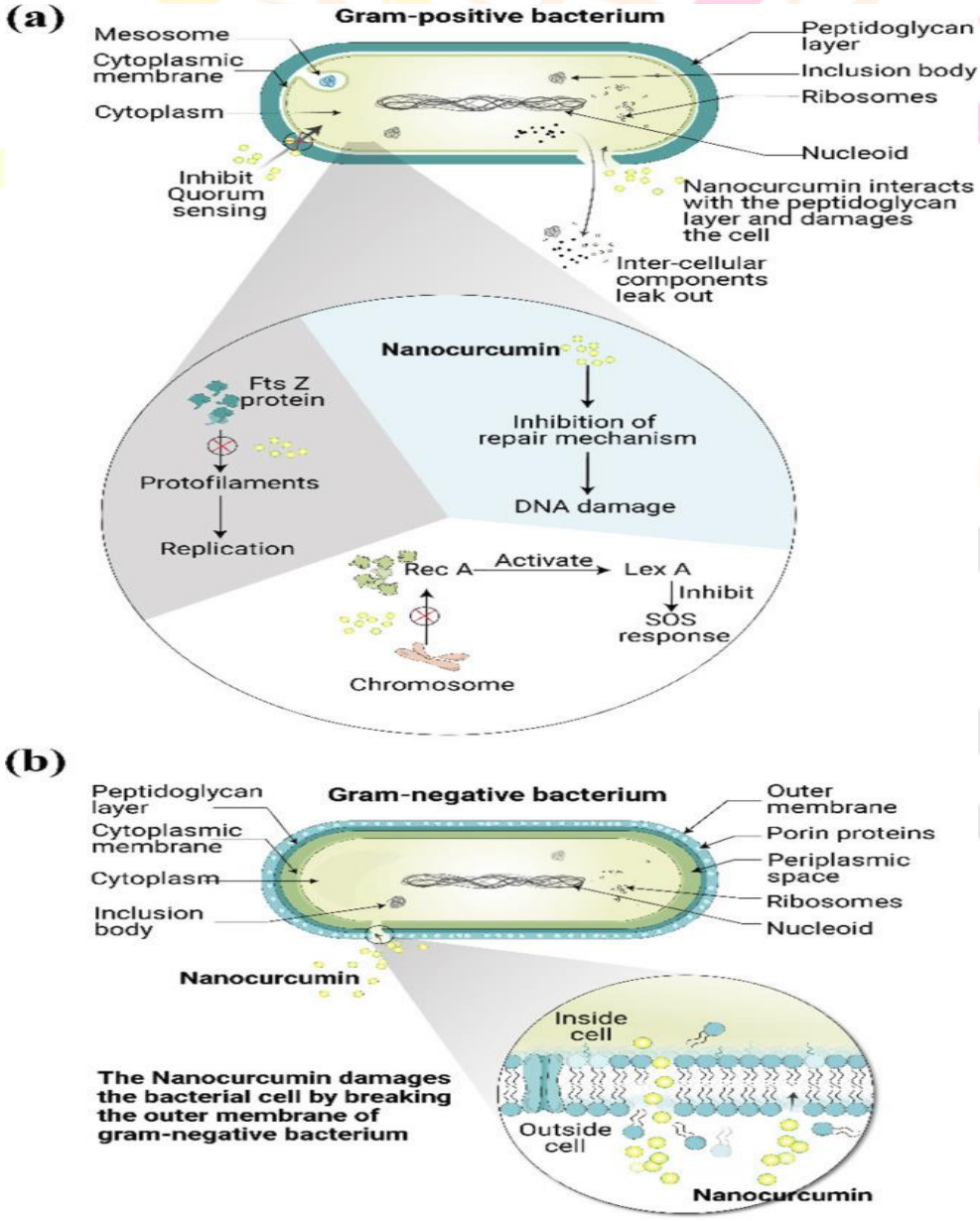

Figure.o. The above figure (a). showing interaction of Nanocurcumin with gram positive bacteria (b) showing interaction of Nanocurcumin with gram Negative bacteria.

REFERENCES

[1] Akram M, Uddin S, Ahmed A, Khan U, Hannan A, Mohiuddin E, Asif M. *Curcuma longa* and curcumin. *Rom. J. Biol. Plant Biol.* 2010, 55, 65–70.Search in Google Scholar
[2] Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: biological actions and medicinal applications. *Cur. Sci.* 2004, 87, 44–53.Search in Google Scholar
[3] Nawaz A, Khan GM, Hussain A, Ahmad A, Khan A, Safdar M. Curcumin: a natural product of biological importance. *Gomal Uni. J. Res.* 2011, 27, 07–14.Search in Google Scholar
[4] Araujo CA, Leon LL. Biological activities of *Curcuma longa* L. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro.* 2000, 96, 723–728.Search in Google Scholar
[5] Aggarwal BB, Bhatt IB, Ichikawa H, Ahn KS, Sethi G, Sandur SK, Natarajan C, Seeram N, Shishodia S. Curcumin biological and medicinal properties. In *Turmeric: The Genus Curcuma*, Ravindran PN, Nirmal Babu K, Sivaraman K, Eds., CRC Press: New York, 2007, pp. 297–368.Search in Google Scholar
[6] Beevers CS, Huang S. Pharmacological and clinical properties of curcumin. *Biol. Targets Ther*. 2011, 1, 5–18.Search in Google Scholar
[7] Bhowmik D, Chiranji B, Sampath KP, Chandira M, Jayakar B. Turmeric: a herbal and traditional medicine. *Arch. Appl. Sci. Res.* 2009, 1, 86–108.Search in Google Scholar
[8] Naz S, Jabeen S, Manzoor S, Aslam F, Ali A. Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. *Pak. J. Bot.* 2010, 42, 455–462.Search in Google Scholar
[9] Damales AC. Potential uses of turmeric (*Curcuma longa*) products as alternative means of pest management in crop production. *Plant Omics J.* 2011, 4, 136–141.Search in Google Scholar
[10] Sharma RA, Geschar AJ, Steward WP. Curcumin the story so far. *Eur. J. Cancer* 2005, 41, 1955–1968.10.1016/j.ejca.2005.05.009Search in Google Scholar PubMed