FABRICATION, CHARACTERIZATION AND *INVITRO* PHARMACOLOGICAL EVALUATION OF CURCUMIN NANOPARTICLES

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ABSTRACT:

Aim: This study was aimed to fabricate curcumin nanoparticles using grape seed oil and evaluate them.

Background: Curcumin, a polyphenol, has been shown to target multiple signaling molecules while also demonstrating activity at the cellular level, which has helped to support its multiple health benefits in inflammatory conditions, metabolic syndrome, pain, oxidative stress, and many more. Despite of its reported benefits, one of the major problems with curcumin is its poor bioavailability, which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination.

Objective: The objective of this study was to develop a novel curcumin-loaded nanoparticles using grape seed oil, characterization and evaluation of its invitro pharmacological activity.

Method: Curcumin nanoparticles were prepared using high-pressure homogenizer method followed by lyophilization to obtain a freeze-dried product. The impact on the particle size, thermal properties, encapsulation efficiency, and loading capacity of the developed nanoformulations will be studied. Additionally invitro anti-inflammatory and antimicrobial studies will be performed on the prepared formulation.

Results: The results showed that the prepared nanoformulation showed a uniform nano-sized spherical shape with the mean diameter of 36.08 nm. The entrapment efficiency and drug loading were 96.97% and 10.74%, respectively. Moreover, in vitro antioxidant, anti inflammatory studies shows protective effect of the developed nanoformulation against oxidative stress and inflammation process. Also the antimicrobial study of the nanoformulation against various gram positive and negative stains shows that the developed formulation can inhibit the growth of various microorganisms.

Conclusion: Hence the developed nanoformulaion characterization studies prove it to be an ideal and also show a promising actions against oxidative stress, inflammation process and antimicrobial actions.

Keywords: Curcumin, Grape seed oil, High pressure homogenizer, lyophyllization, anti inflammatory.

Introduction

The versatility in nanomedicine field is evolving with throughout the time and vast investigations are being performed by the researchers worldwide. The system of nano-drug delivery can be classified as polymeric systems, such as nanocapsules, nanofibres, nanospheres, nanodiscs, and lipid-based systems, such as niosomes, phytosomes, liposomes, transferosomes, micelles, solid lipid nanoparticles, and nanostructured lipid carrier systems[(1)].

Over the past years, many nanoparticulate methods have been researched to address the issues with herbal bioactives. Among them, lipid based nanocarriers such as solid lipid nanoparticles (SLN) and nanolipid carriers (NLC) have a number of benefits over other

nanoparticles, including increased stability, increased cell permeability, higher drug entrapment, biodegradability, and biocompatibility. NLC, also referred to as a second generation lipid nanocarrier, is the next iteration of the SLN. Unlike SLN (solid lipid core stabilised with surfactant), NLC has an irregular lattice structure and is structurally made up of a special combination of solid and liquid lipids [2, 3]. The imperfect structure allows greater space for housing the drug load and, as a result, has higher entrapment efficiency. It has many advantages over SLN, including improved drug loading and trapping, increased stability, and reduced drug expulsion during storage. Additionally, when compared to onther nanoformulations, it is more biocompatible, and less hazardous due to the utilisation of natural lipids or their derivatives. NLC considerably increases the solubility of lipophilic medicines and makes it easier for medication to be absorbed [4].

The solid, liquid lipids, surfactants, and surface modifiers are the main components of lipid nanoparticles. The Solid lipid serves as the source of matrix formation and make up the solid lipid core. The lipophilic excipients excipients such as lipid liquid are employed to integrate the solid lipid core and lessen its crystallinity. In the formulation process the active ingredients are mainly dissolved in synthetic oils, among the two lipids [5, 6]. The lipid component of the formulation influences stability, entrapment efficiency, drug-loading capacity, and the controlled release behaviour. These lipids are approved as generally regarded-as-safe (GRAS) substances and are physiologically well tolerated. However, the important criterion to choose an acceptable lipid is the drug solubility in lipids. The polymeric substance called surfactant is the third component frequently employed to stabilise the lipid nanoparticles, ensure uniform particle size distribution and inhibit particle aggregation. Surfactants are essential in nanoparticle formulation because they affect a number of properties such as viscosity, and water solubilising capacity. Also they provide formulation stability,aaids in dispersibility and inhibit particle agglomeration. Surfactants are amphiphiles that lowers the surface tension, making it easier for particle distribution between two phases. Based on the chosen lipid component, surfactants are selected because they should have physiochemical compatibility with each other. The route of drug delivery and the surfactants hydrophilic–lipophilic balance (HLB) are the additional variables that affect the choice of surfactant [7, 8, 9].

The rhizomes of the plant Curcuma longa are the source of curcumin, a strong phytochemical found in turmeric. The regular use of this phytochemical with improved bio distribution provides substantial biological activity as anti-inflammatory, antioxidant, anti-cancerous, antimicrobial [10, 11, 12].

Curcumin is a promising candidate for its use in the treatment of various diseases due to its biological activity and pharmacological safety in high doses, even at 12g per day. Curcumin's therapeutic use is severely constrained by its poor bioavailability, which is caused by both its low solubility and instability. Vast research on curcumin on its pharmacokinetic parameters showed that it has limited absorption, quick metabolism, and rapid elimination [13, 14].

Various studies focussing on metabolism and distribution of curcumin demonstrated a very low or no availability of curcumin to the cells and the target tissues. Poor absorption, rapid metabolism and elimination with poor activity of the metabolically breakdown products are the main contributors for this [15, 16, 17]. The problem of poor oral bioavailability has been addressed using a variety of approaches including nanosized curcumin formulation, lipid-based drug delivery with liposomal curcumin encapsulation, encapsulation of curcumin in nano starch, phospholipid and curcumin complex formation and combination of metal and curcumin chelation [18, 19]

The nutritional benefits of the raw and dried fruit, wine and the therapeutic benefits of derivatives such as peel and seed extracts of *Vitis vinifera* L. ssp. sativa grapes are of worldwide interest [20, 21]. For example, grape seed extract (both aqueous and alcoholic) has a high antioxidant potential; its positive effects include the modulating the expression of antioxidant enzyme, protection against oxidative cell damage, antiatherosclerotic and anti-inflammatory effects, and protective action against some cancers, in both humans and animal models [22, 23, 24].

Grape seed constitutes about 8%–20% of oil on dry basis. Due to its high concentrations of lipophilic constituents like vitamin E, unsaturated fatty acids, and phytosterols as well as hydrophilic components like phenolic compounds, grape seed oil has gained popularity as functional food product [25, 26]. The composition of grape seed oil depends on variety of environmental factors and the level of seed maturity. The interest in using grape seed oil in culinary preparations has grown as a result of its organoleptic qualities, which include its scent and pleasant flavour [27, 28].

The flavanoids, carotenoids, phenolic acids, tannins, and stilbenes are the phenolic compounds found in grape seed oil. (Garavaglia et al) Additionally, it contains 50-300mg of gallic acid equivalent per kg of phenols, which have been linked to a variety of biological processes but are best known for their antioxidant effects. Grape seed oil contains trans resveratrol, procyanidin B_1, catechins, and epicatechins as its primary polyphenols [29, 30].

Linoleic acid (LIA), which makes up between 66.0% and 75.3% of all fatty acids (FA) in cold pressed grape seed oils, is the most prevalent FA. LIA is a PUFA (poly unsaturated fatty acid) that has been linked to the improvement of human health. The percentage of PUFA in grape seed oil is high, ranging from total 85%–90%.11. Additionally, grape seed oil has a significant amount of oleic acid, a monounsaturated fatty acid (MUFA), and less of the saturated fatty acids. Each grape variety has a unique FA composition, as does the oil it produces. Oleic acid, a monounsaturated FA (MUFA), is also largely found in grape seed oil, and saturated fatty acids (SFAs) are present in lower quantities. Each grape variety and its oil have a different FA composition [31].

There is a specific tocopherol and tocotrienol present in grape seed oil. Tocopherols including α-, β-, γ-, and δ are found in grape seed oil and also have a powerful antioxidant activity. Tocotrienols (unsaturated forms of vitamin E) are more plentiful in grape seed oils.

Vitamin E supports the health benefits of grape seed oil due to its high antioxidant activity, neuroprotective and, antitumoral capacity. Due to this, it has been suggested that grape seed oils be used to slow down the ageing process and stave off the development of several chronic illness. Phytosterols, another group of lipophilic substances primarily present in grape seed oil, may inhibit the release of pro inflammatory mediators by oxidised low density lipoprotein stimulated macrophages during oxidative stress and eicosanoid production [32].

Grape polyphenols have been found to have cardioprotective, antiarthritic, anti-diabetic, anti-cancer, anti-obesity, anti-neurodegenerative and anti-microbial properties. These effects are mediated through modulation of signal transducers, such as SIRT-1 gene induction, and inhibition of NFkappaB and mTOR gene expression, among other inflammatory genes (COX-2, MMPs). In the past few years there have been a substantial number of research on the binding of polyphenols to drug carriers, particularly the nanoscale conjugate development. The effective usage of polyphenols requires substitution at higher concentrations than usual. Resveratrol, a key component of grape seed oil, is essential for the creation of anticancer nanobioconjugates that contain polyphenols (e.g., dendrimers, polymer nanoparticles, liposomes, nanotubes, micelles, etc.) [33, 34]

Materials and methods

Materials

Curcumin and grape seed oil were obtained as a gifted sample from Rumi herbals, Erode. Stearic acid, Tween 80, soya lecithin, was purchased from Hi Media (Mumbai, India). The rest of the reagents were all of analytical grade.

Preparation of Curcumin loaded solid lipid nanoparticles

Based on a previous literature, two distinct lipids, stearic acid (a solid lipid) and grape seed oil (a liquid lipid)—were chosen for the current study. The surfactant and stabiliser used were tween 80 and soy lecithin (SL), respectively. The process of hot high-pressure homogenization (HPH) was used to formulate lipid nanoparticles. In a nutshell, a temperature of 85±0.5^{0}C was used to melt the lipid phase that contained curcumin, stearic acid, and grape seed oil. Pre-emulsion was prepared by mechanically agitating the hot aqueous surfactant (Tween-80 and SL) with the above melted lipid phase for 20 minutes at 1500 rpm at 85±0.5^{0}C. The resulting pre-emulsion was then passed through HPH at 600 bar pressure for four cycles. Ultimately, the resulting hot o/w nanoemulsion was cooled to 4±0.5 ^{0}C, for recrystallization of the lipid and the curcumin loaded nanoparticles were generated [35, 36].

Centrifuging the finished product at 45,000 rpm for 35 minutes at room temperature allowed the lipid nanoparticles to be separated from the product. The material was then lyophylized using 5% mannitol, as cryoprotectant. The sample vials were sealed with rubber closures after freeze drying for 28 hours [37, 38].

Characterization of nanoparticles:

Evaluation of particle size (PS), polydispersity index (PDI) and zeta potential (ZP):

Malvern Nano ZS® Zetasizer (Malvern Nano ZS®) was used to determine the mean PS and PDI of the produced curcumin nanopartilces using dynamic light scattering. The test samples were diluted with double-distilled water until the mixture had a faint opalescent appearance. The test samples were diluted with double-distilled watertill the solution appeared weakly opalescent [39, 40]

MORPHOLOGY OF NANOPARTICLES:

Scanning electron microscopy (SEM):

The incidence of an accelerated electron beam on the sample is the foundation of scanning electron microscopy (SEM). The morphology of the curcumin nanoparticles was investigated using a scanning electron microscope and an accelerating voltage of 20 kV. Samples were prepared by placing the nanoparticles on to an aluminum specimen stub, dried overnight, and sputter coated with gold prior to imaging [41].

Differential scanning calorimetry:

Thermal analysis was conducted using a differential scanning calorimetry (DSC). The instrument was calibrated with indium. The sample, weighing 2 mg, was analyzed in sealed and, pin-holed standard aluminum pan, with a heating rate of 10^0 C/min from 30 ^{0}C to 300 ^{0}C. During measurement, the sample cell was continuously purged with nitrogen at a flow rate of 40 ml/min [42].

UV Estimation:

A 10mg of the formulated lipid nano curcumin will be accurately weighed and transferred in a 100ml volumetric flask. Methanol will be added up to the mark to obtain a 100µg/ml Stock solution. From Stock solution a concentrations of 5, 10, 15, 20, and 25µg/ml, respectively were prepared. The prepared dilution will be scanned in UV spectrophotometer in the range of 420nm with methanol as blank. Least square regression analysis will be done to obtain linearity range [43].

Entrapment efficiency and drug loading:

Entrapment efficiency is an essential parameter for characterizing lipid nanoparticles. This parameter provides information on the drug that was entrapped. The entrapment efficiency of prepared nanoparticles was determined by the centrifugation method. A formulation (containing drug equivalent to 10mg of drug) was centrifuged at 10,000rpm for 40min in high centrifuge to collect supernatant liquid. The collected liquid was filtered to measure the quantity of free drug concentration after suitable dilution with the fresh phosphate buffer saline of pH 7.4. The absorbance was measured at 430 nm in a UV-visible spectrophotometer. The entrapment efficiency was calculated using the formula:

E.E= (Amount of total drug - Amount of drug in aqueous phase)/ Amount of total drug X100

Loading capacity/drug loading (DL) represents the percentage of drug incorporated in the lipid nanoparticles in relation to the total weight of the lipoidal phase (i.e. Lipid + drug). For this, the curcumin from lyophilized powder was extracted with DMSO and methanol. The curcumin content was analyzed Spectrophotometrically (UV 1700, Shimadzu, Kyoto, Japan) at 423 nm, against the DMSO and methanol mixture as a Blank [44].

$$\% \text{ DL} = \frac{\text{Amount of drug added in NLC}}{\text{Amount of drug added + amount of excipients added}} \times 100$$

Fourier transforms infrared spectroscopy (FTIR):

Infrared Spectra of pure curcumin and the formulated nanocurcumin particles were scanned on a FTIR spectrophotometer with the frequency of 500–4000 cm $^{-1}$. The sample was prepared by mixing the sample and KBr pellets at the ratio of 1:50 [45].

Antimicrobial activity:

Determination of Zone of Inhibition: A well-diffusion method on agar plates was used to analyze the antibacterial activity against test strains. A total of 100 µL of diluted inoculum from organism suspensions was spread on the surface of the plates and allowed to solidify. Under aseptic condition three wells were cut out with the help of a well borer on the agar medium. The wells are filled with 400 µg of nanocurcumin and curcumin solutions and DMSO as the control. The plates were incubated for 24 h at 37 ^{0} C. ed at 37 0 C for 24 hours. The antimicrobial activity was assessed by measuring the diameter of the transparent inhibitory zone against test bacteria [46].

In-vitro **Anti-inflammatory Activity:** As per previously reported protocol the reaction mixture contained 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract. As a control, the same volume of double-distilled water was used. The mixtures was then incubated at 37°C ± 2°C for 15 min and then heated at 70°C for 5 min. After cooling, the absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium was be used as standard. The percentage inhibition of protein denaturation was calculated using the following formula [47]:

$$\% \text{ inhibition} = \{(\text{Abs of control} - \text{Abs of test})/\text{Abs of control}\} \times 100$$

Results:

		Size (r.nm):	% Intensity	Width (r.nm)
Z-Average (r.nm): 36.08	Peak 1:	61.69	94.0	49.33
PdI: 0.466	Peak 2:	1892	6.0	579.0
Intercept: 0.640	Peak 3:	0.000	0.0	0.000

Result quality : Good

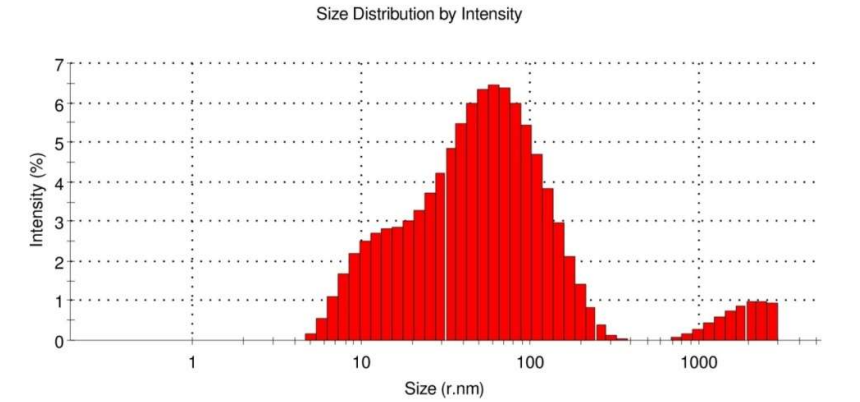

Fig: 1 Particle size distribution and PDI of the nanoformulation.

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 14.2	Peak 1:	17.2	90.5	15.5
Zeta Deviation (mV): 18.4	Peak 2:	-20.7	9.5	6.47
Conductivity (mS/cm): 2.00	Peak 3:	0.00	0.0	0.00

Result quality : See result quality report

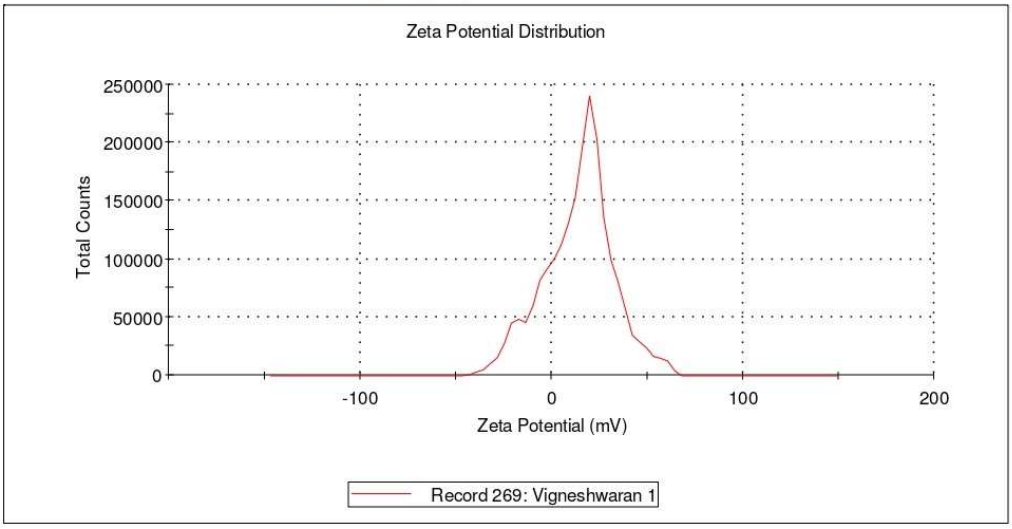

Fig: 2 Zeta potential of the nanoformulation.

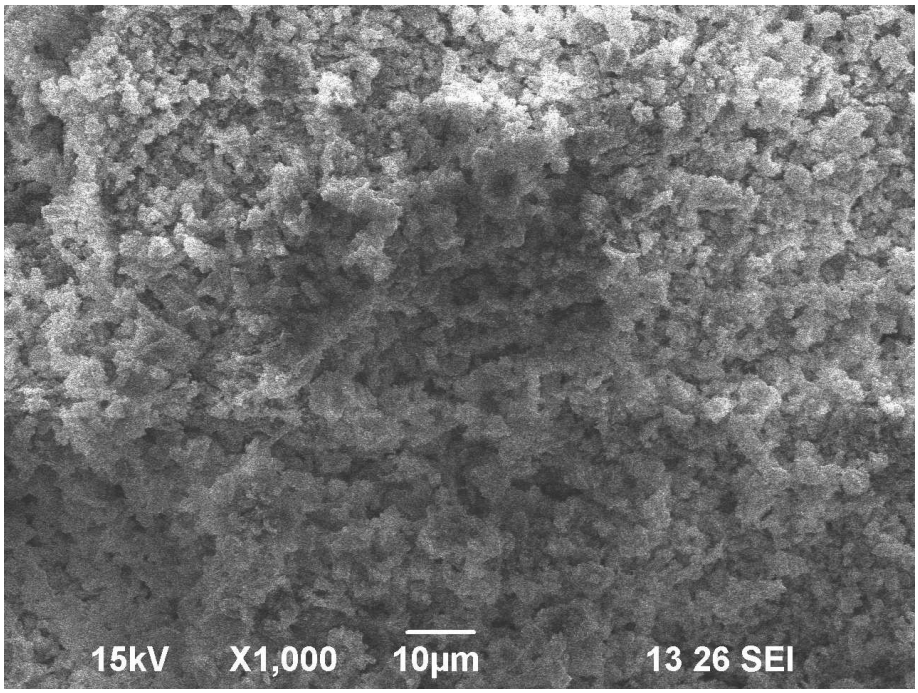

Fig: 3 SEM image of the prepared nanoparticles.

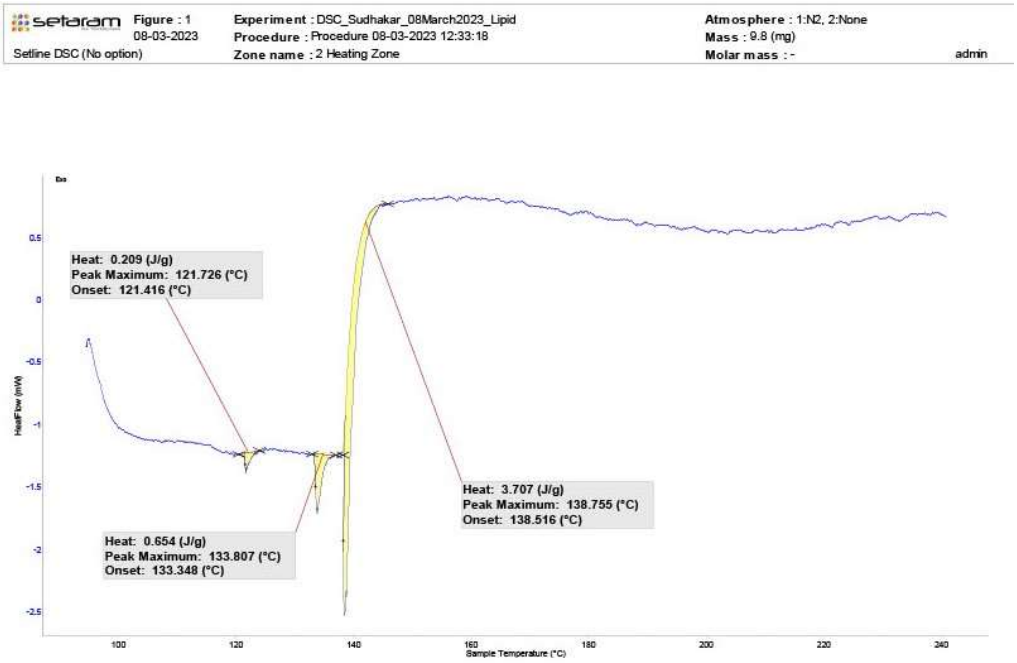

Fig: 4 DSC Thermogram of curcumin nanoparticles.

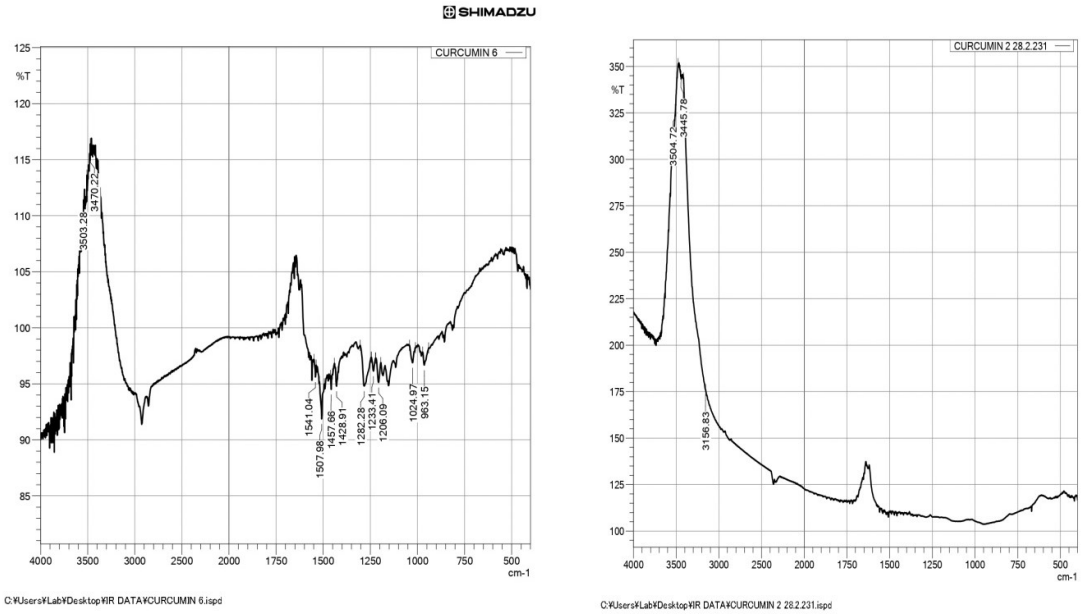

Fig: 5 FTIR spectrum of Curcumin and Nanocurcumin formulation.

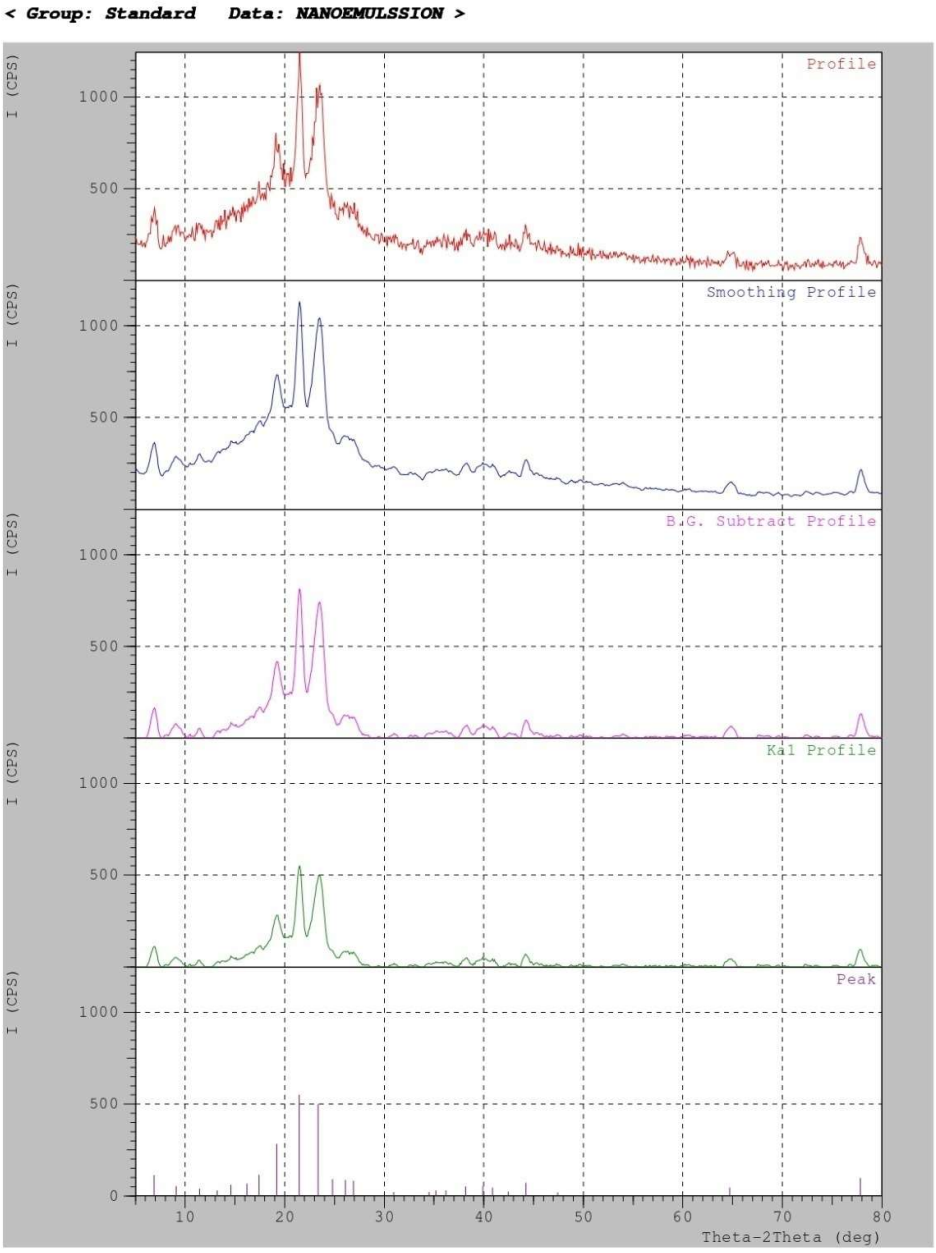

Fig: 6 UV absorbance for curcumin Nanoparticles

Concentration mcg/ml	Absorbance
5	0.12
10	1.23
15	2.24
20	3.46
25	4.53

Table: 1 UV absorbance for curcumin

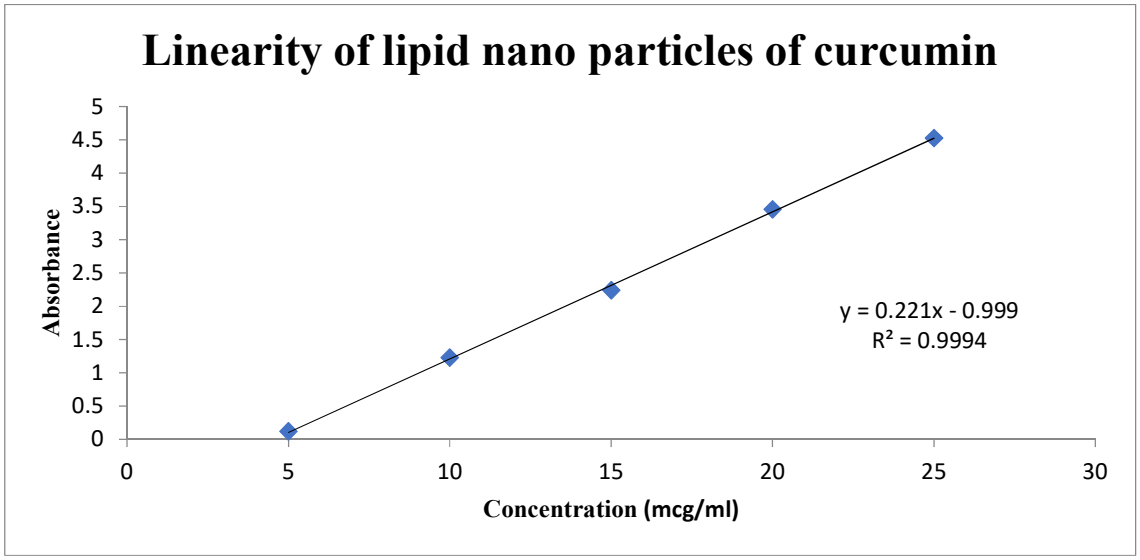

Fig: 7 Linearity of lipid nano particles of curcumin

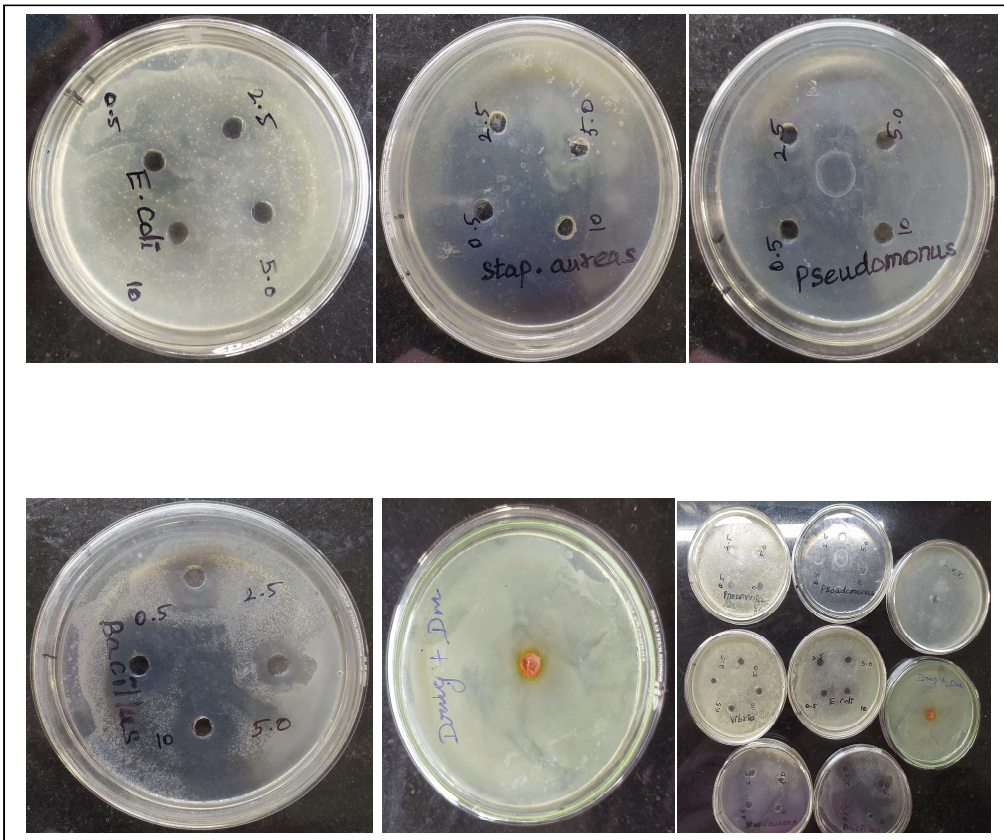
0.5
2.5
E.Coli
5.0
10
2.5
5.0
0.5
10
stap. aureas
2.5
5.0
0.5
10
pseudomonus
0.5
2.5
Bacillus
10
5.0
Dmf + Dm

Fig: 8 Anti-bacterial Study of nanocurcumin

Organism	Zone of inhibition in mm				
	Pure curcumin 10 µg/ml	Lipid nanoformulation in µg/ml			
		0.5	2.5	5	10
E.coli	16.66±2.49	6.3±0.94	16+±1.6	21.3±1.24	24.3±1.24
S. aureus	18.66±3.29	5.66±0.47	13.66±1.24	20.33±0.94	23.66±0.47
Pseudmonas	19±2.94	6.33±1.24	11.33±1.31	21.5±1.69	23.33±1.7
Bacillus	18.33±3.39	6.66±2.05	12±2.16	18.66±2.62	21±1.63
Vibrio	16.33±2.86	5±1.41	9.33±2.05	19.76±2.49	22.71±2.94
Pneumonia	11.66±2.05	5.33±2.05	7.66±1.24	9.33±1.24	15.33±1.24

Table: 2 Zone of inhibition of Anti-bacterial study

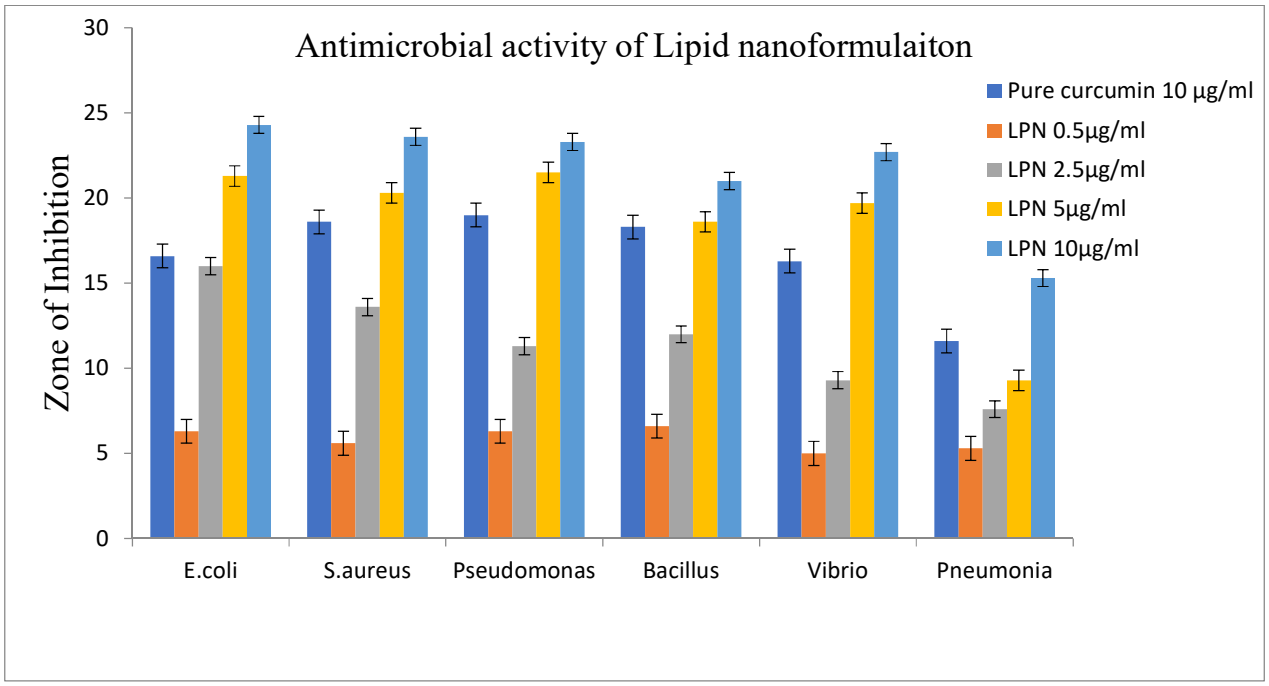

Fig: 9 Antimicrobial activity of Lipid nanoformulation

S.No	Concentration µg/ml	% inhibition of standard	% inhibition of lipid nanoformulation
1	0	0	0
2	50	30.68±1.33	29.2±0.64
3	100	42.6±0.98	41.03±1.45
4	200	57.3±0.96	55.5±1.18
5	400	68.2±1.02	66.16±1.06

Table: 3 Concentration Dependent inhibition of Protein Denaturation

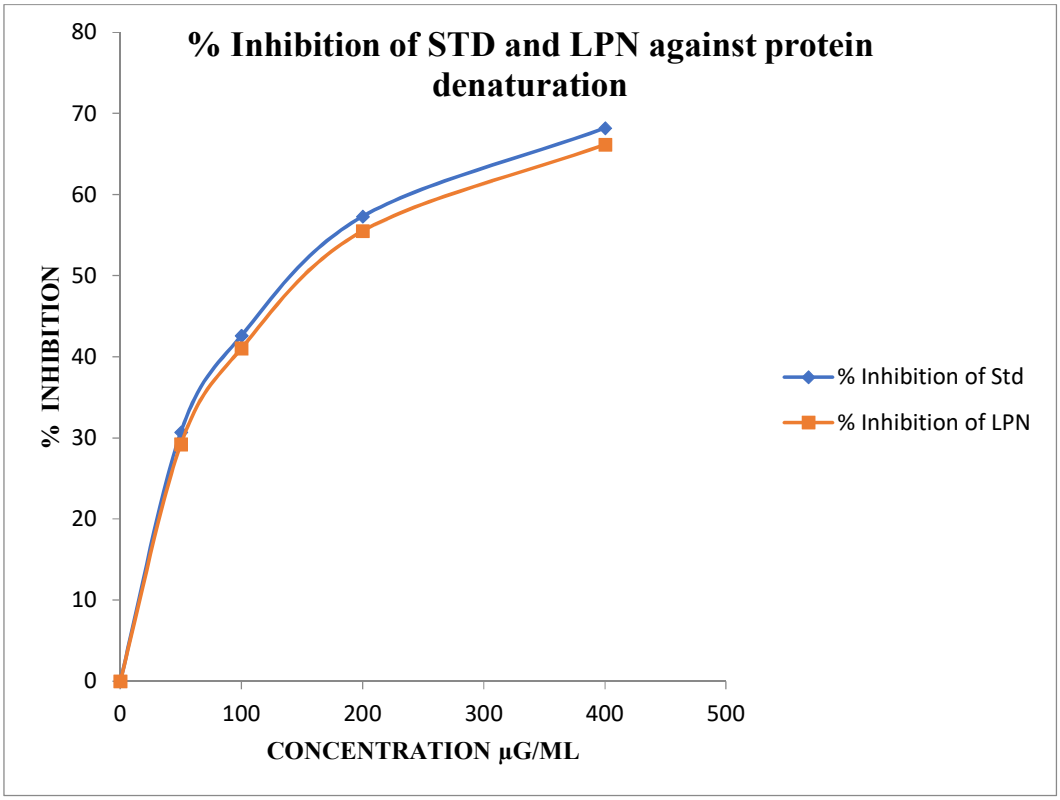

Fig: 10 Percentage Inhibition of STD and LPN against protein denaturation

Discussion:

The hot pressure homogenization process was successfully employed to formulate curcumin loaded nanoparticles [48, 49]. Stearic acid and grape seed oil were chosen as solid matrix and liquid lipid respectively, for the synthesis of curcumin nanoparticles [50, 51, 52]. The surfactants chosen were Tween 80 and soy lecithin. The high pressure homogenization method was easy and quick, and no organic solvents were used in formulation development. The produced nanoparticles were subjected to lyophyllization and the nanotized curcumin was found to be stable both physically and chemically, readily dispersible in water, and capable of being stored at room temperature without any decomposition or aggregation [53, 54].

Particle size and Polydispersity Index (PDI) significantly impact the stability, release rate and solubility.Additionally, the bioavailability of encapsulated compounds increases with decreasing particle size. The Particle size and PDI depends on the production condition, component ratio and lipid phase viscosity. The Particle size analysis revelaed that the nanoparticles were in the range of 10-300 nm [Figure:1] with a mean size of 36.08 nm. Polydispersity index displays the range of particle sizes distribution between 0-1. The PDI value between 0.1-0.25 indicates a relatively narrow distribution and while values higher than 0.5 indicates a broad distribution [55, 56].

The electrical charge on the surface of the NLCs is represented by the zeta potential. The greater the ZP value, the more likely the formulation said to be stable because the charged particles repel one another and so counteract the tendency to aggregate.

It is currently accepted that greater the ZP values, either positively or negatively charged, mean that dispersion will have greater long-term stability. The zeta potential is an important factor in understanding the state of the nanoparticle surface. It also foretells the nanoparticles long term stability and plays a crucial role in defining how they interact with the cell membrane invivo. The zeta potential of nanotized curcumin [Figure:2] was found to be 14.2±18.4 mV, indicating high stability of the formulation.

Morphological investigation of freeze dried formulation was carried by taking scanning electron microscopy (SEM) images. They were found to be ovoid shape (Figure 8). The formulated nanocurcumin were examined for crystallinity and purity using XRD, a potent analytical method for determining the crystalline phases and purity of sample particles. The results demonstrated that the synthesized nanocurcumin is amorphous. As per previous literatures Pure CRM reflections were precise in line. The Lyophilized CRM-loaded nanoparticles in Figure 3 had low intensity diffractograms that illustrated the amorphous nature of NLCs.

DSC was a fundamental technique for analysing the crystallization or amorphous state of drug in the nanoformulation by varying the temperature and energy at phase transition. [Figure 4] shows the DSC curve of lyophilized curcumin nanoparticles. The thermogram revealed a melting peak of curcumin nanoformulation at around 138^0 C. The disappearance of the endothermic peak of lyophyllized nano curcumin suggests that the drug was molecularly dispersed in the lipid matrix and converted to an amorphous state from the crystalline state [(57)].

The Fourier transform infrared spectroscopy (FTIR) spectra of curcumin and nanocurcumin were scanned at the midinfrared region (4000−400 cm^{-1}), and the spectra are depicted in [Figure: 5]. The obtained bands vibrations of the functional groups present in curcumin and nanocurcumin respectively. The intense band at wavenumber 3503 cm^{-1} corresponds to the stretching vibration of hydrogen-bonded O−H present in curcumin. Bands at 1457 and 1428 cm^{-1} correspond to the aromatic stretching vibrations of the benzene ring. The intense characteristic band centered at 1507 cm^{-1} corresponds to the stretching vibration of the conjugated carbonyl (C=O). The bands centered at 1282 cm^{-1} and centered at 1206 cm−1 originate from the stretching vibrations of C sp2− O and C sp3−O bonds, respectively (28). Considering nanocurcumin, the broad, intense band at wavenumber 3445 cm^{-1} corresponds to the stretching vibration of hydrogen-bonded O−H present in nanocurcumin. The intense characteristic band centered at 1600 cm^{-1} corresponds to the stretching vibration of the conjugated carbonyl (C=O).

Linearity of an analytical method is its ability, within the given range, to obtain test results that are directly, or through mathematical transformation, proportional to concentration of analyte [Figure: 6]. Excellent linear correlations were obtained between absorbance and concentration in the selected range of 5 – 25mcg/ml with a correlation coefficient of 0.9994 tabulated in table 1 [Figure: 7].

The EE measurement of curcumin loaded nanoparticles resulted in a relatively high yield, which was 96.97%. From this test, the average DLC was 10.74%. In the disc diffusion method, pure curcumin, and the curcumin loaded lipid nanoparticles were placed on the agar plates, which contain uniformly selected, swabbed, pure bacterial strains. Then, the plates are incubated under suitable conditions according to the chosen bacterial strain. The disk constituents diffuse into the agar medium from the disks. If the concentration of the drug is effective against bacteria, there will not be growth of any colonies. These are the zone of inhibition. The diffusion rate of the antimicrobial compound through the agar depends on the diffusion and solubility of the drug in the agar, and the diffusion depends on the size of the antimicrobial compound [Figure:8]. The curcumin is not soluble in water. Due to reduction in size of nanocurcumin and enhanced water solubility, it can diffuse through the agar medium at a higher rate when compared to curcumin bulk particles. As a result nanocurmin exhibits a considerably greater zone of inhibition shown in tabe 2. In our experiments, curcumin, and lipid nanoparticles, were placed on the same agar plate, thereby eliminating any potential inaccuracies that may be caused by the depth variation of the agar medium. Dimethyl sulfoxide (DMSO) was used to dissolve curcumin and nanocurcumin in the antibacterial study because both of them have very high solubility in DMSO [58, 59]. The results were represented graphically in table 2.

The response of living tissues to stimuli elicited by inflammatory agents, such as physical trauma, heat, microbial infections, and toxic chemical irritations, is known as inflammation. Cells' reactions to inflammation can result in pathological symptoms as redness, heat, swelling, and discomfort as well as compromised physiological processes. Numerous disorders, including cancer, arthritis, and stroke, have inflammation as a contributing factor in their pathogenesis. Protein denaturation causes the inflammatory response, which in turn causes a variety of inflammatory illnesses, including arthritis. According to literature, tissue damage during life might be related to denaturation of the proteins that make up cells or the material between the cells. Therefore the ability of a compound to avert the protein denaturation implies apparent potential for an anti-inflammatory efficacy. The curcumin nanoparticles exhibited an anti-inflammatory activity as presented in [Table 3]. The agents that can inhibit protein denaturation, would possiblly a potent candidate for anti-inflammatory drug. With this in mind, an in vitro test was conducted as a preliminary check to screen anti-inflammatory property. The protein denaturation bioassay of curcumin nanoparticles was done with a wide range of dose concentrations. Diclofenac sodium (at the concentration range of 50- 1000 µg/ml) was used as the standard drug, which also exhibited a concentration dependent inhibition of protein denaturation [Table 3]. The increased absorbance in both the test and the standard drug indicates an increase in protein stabilizing activity (denaturation is inhibited) with increased dose [Figure: 10]. Therefore, it is evident

that the produced curcumin nanoparticles have strong anti-inflammatory action and may potentially compare to that of the normal medication [60, 61].

Conclusion:

In this work, a simple and robust strategy of curcumin nanoparticles was developed to avert the autoxidation of curcumin and to enhance its cellular uptake. Grape seed oil perfectly retained the structural integrity of curcumin and the formulation also protected curcumin against autoxidation possibly by scavenging of $O2^{\cdot -}$, $RO^{\cdot}$ and $OH^{\cdot}$ by the antioxidants present in Grape seed oil. The fabricated formulation possesses high entrapment efficiency, narrow size distribution and controlled drug release behaviour using binary lipids. The formulation showed a strong antimicrobial activity against different Gram-positive, Gram-negative micro organism, *invitro* anti inflammatory activity. Further studies are planned to evaluate the formulation for an innovative anti-inflammatory in various animal models. However, extensive studies are needed to investigate and demonstrate the above said activities of curcumin grape seed oil nanoparticles.

Reference:

1. R.G. Madane, H.S. Mahajan. Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration: design, characterization, and in vivo study. Drug Deliv. 2016:23 (4); 1326–34.
2. Khosa. S, Reddi. R.N. Saha. Nanostructured lipid carriers for site-specific drug delivery. Biomed. Pharmacother. 2018: 103; 598–613.
3. H. Barabadi, H. Vahidi, K. Damavandi Kamali, M. Rashedi, O. Hosseini, M. Saravanan. Emerging theranostic gold nanomaterials to combat colorectal cancer: a systematic review. J. Clust. Sci. 2020: 31 (4); 651–58.
4. Esposito. E, Sguizzato. M, Drechsler. M, Mariani. P, Carducci. F et al., Lipid nanostructures for antioxidant delivery: A comparative preformulation study. Beilstein J. Nanotechnol. 2019: 10; 1789–801.
5. K.V. Krishna, G. Wadhwa, A. Alexander, N. Kanojia, R.N. Saha, et al., Design and biological evaluation of lipoprotein-based donepezil nanocarrier for enhanced brain uptake through oral delivery. ACS Chem. Neurosci. 2019: 10 (9); 4124–35.
6. Beloqui. A, Solinis. M. A, Rodriguez Gascon. A, Almeida. A. J, Preat. V. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. Nanomedicine. 2016: 12(1); 143–61.
7. Subramanian. P. Lipid-Based Nanocarrier System for the Effective Delivery of Nutraceuticals. Molecules. 2021: 26; 2-26.

8. S.N. Kanojia, N. N. Gupta, S. Singh. Applications of nanostructured lipid carriers: recent advancements and patent review. Biointerface Res. Appl. Chem. 2021: 12 (1); 638–52.
9. M. Agrawal, S. Saraf, S.K. Dubey, A. Puri, R.J. Patel. Recent strategies and advances in the fabrication of nano lipid carriers and their application towards brain targeting. J. Control. Release. 2020: 321; 372–415.
10. Pawar. H. A, Gavasane. A. J, Choudhary. P. D. A Novel and Simple Approach for Extraction and Isolation of Curcuminoids from Turmeric Rhizomes. Adv. Recycl. Waste Manag. 2018: 6; 21-25.
11. Shivarkar Rahul Shashikant, SB Bhise, V Rama Mohan Gupta. Standardization and Quantification of Curcumin Present in Curcuma Longa Extract Using HPLC. International Journal of Phytopharmacology. 2017; 8(3): 117-22.
12. Reddy. A.S, Lakshmi. B.A, Kim. S, Kim. J. Synthesis and characterization of acetyl curcumin-loaded core/shell liposome nanoparticles via an electrospray process for drug delivery, and theranostic applications. Eur. J. Pharm. Biopharm. 2019: 142; 518–30.
13. Behbahani. E.S, Ghaedi. M, Abbaspour. M, Rostamizadeh. K, Dashtian. K. Curcumin loaded nanostructured lipid carriers: *In vitro* digestion and release studies. Polyhedron. 2019: 164; 113–22.
14. Rabima. A, Oktamauri. Characterisation and cytotoxicity assay of curcumin nanostructured lipid carrier on HeLa cells, IOP Conf. Ser. Earth Environ. Sci. 2021: 667 (1); 13-18.
15. Neves. A, van der Putton. L, Queiroz. J, Pinheiro. M, Reis. S. Transferrin-functionalized lipid nanoparticles for curcumin brain delivery. J. Biotechnol. 2021: 331; 108–117.
16. Ban. C, Jo. M, Park. Y.H, Kim. J.H, Han. J.Y, et al., Enhancing the oral bioavailability of curcumin using solid lipid nanoparticles. Food Chem. 2020: 302; 125-28.
17. Zheng. B, Zhang. X, Peng. S, McClements. D. J. Impact of curcumin delivery system format on bioaccessibility: nanocrystals, nanoemulsion droplets, and natural oil bodies. Food Funct. 2019: 10; 39–49.
18. Rohini G. Madane, Hitendra S. Mahajan. Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration: design, characterization, and invivo study. Drug Delivery. 2016: 23(4); 1326-34.

19. Ping Chen, Hui Zhang, Shucang Cheng, Guangxi Zhai, Chengwu Shen. Development of curcumin loaded nanostructured lipid carrier based thermosensitive in situ gel for dermal delivery. Colloids and Surfaces A: Physicochem. Eng. Aspects. 2016: 506; 356–62.
20. R. Giacomeli, J.C. Izoton, R.B. dos Santos, S.P. Boeira, C.R. Jesse, S.E. Haas. Neuroprotective effects of curcumin lipid-core nanocapsules in a model Alzheimer's disease induced by β-amyloid 1-42 peptide in aged female mice. Brain Res.2019: 15; 146-53.
21. N. Ahmadi, M.-J. Hosseini, K. Rostamizadeh, M. Anoush. Investigation of therapeutic effect of curcumin α and β glucoside anomers against Alzheimer's disease by the nose to brain drug delivery. Brain Res. 2021 76; 147-57.
22. Garavaglia et al. Grape Seed Oil Compounds: Biological and Chemical Actions for Health. *Nutrition and Metabolic Insights.* 2016:9; 59–64.
23. Gomez Mejia. E, Roriz. C.L, Heleno. S.A, Calhelha. R, Dias. M.I, etal., Valorisation of black mulberry and grape seeds: Chemical characterization and bioactive potential. Food Chem. **2020**: 337; 79-98.
24. Hegedus. I, Andreidesz. K, Szentpeteri. J.L, Kaleta. Z, Szabo. L etal., The Utilization of Physiologically Active Molecular Components of Grape Seeds and Grape Marc. Int. J. Mol. Sci. **2022**: 23; 2-34.
25. Yousefi. R, Parandoosh. M, Khorsandi. H, Hosseinzadeh. N, Madani Tonekaboni. M etal., Grape seed extract supplementation along with a restricted-calorie diet improves cardiovascular risk factors in obese or overweight adult individuals: A randomized, placebo-controlled trial. Phytother. Res. **2021**: 35; 987–95.
26. Asbaghi. O, Nazarian. B, Reiner. Z, Amirani. E, Kolahdooz. F etal., The effects of grape seed extract on glycemic control, serum lipoproteins, inflammation, and body weight: A systematic review and meta-analysis of randomized controlled trials. Phytother. Res. **2020**: 34; 239–53.
27. Ghendov Mosanu. A, Cojocari. D, Balan. G, Patras. A, Lung. I etal., Chemometric Optimization of Biologically Active Compounds Extraction from Grape Marc: Composition and Antimicrobial Activity. Molecules. **2022**: 27; 60-77.
28. Trapani. A, Esteban. M.Á, Curci. F, Manno. D.E, Serra. A, etal., Solid Lipid Nanoparticles Administering Antioxidant Grape Seed-Derived Polyphenol Compounds: A Potential Application in Aquaculture. Molecules. **2022**: 27; 344-56.

29. Castellani, S.; Trapani, A.; Spagnoletta, A.; di Toma, L.; Magrone, T.; Di Gioia, S.; Mandracchia, D.; Trapani, G.; Jirillo, E.; Conese, M. Nanoparticle delivery of grape seed-derived proanthocyanidins to airway epithelial cells dampens oxidative stress and inflammation. J. Transl. Med. **2018**, 16, 140.
30. Trapani, A.; Esteban, M.Á.; Curci, F.; Manno, D.E.; Serra, A.; Fracchiolla, G.; Espinosa-Ruiz, C.; Castellani, S.; Conese, M. Solid Lipid Nanoparticles Administering Antioxidant Grape Seed-Derived Polyphenol Compounds: A Potential Application in Aquaculture. Molecules 2022, 27, 344.
31. Lu, R.H.; Qin, C.B.; Yang, F.; Zhang, W.Y.; Zhang, Y.R.; Yang, G.K.; Yang, L.P.; Meng, X.L.; Yan, X.; Nie, G.X. Grape seed proanthocyanidin extract ameliorates hepatic lipid accumulation and inflammation in grass carp (Ctenopharyngodon idella). Fish Physiol. Biochem. 2020, 46, 1665–1677. [CrossRef] 4.
32. Cerbaro, A.F.; Rodrigues, V.S.B.; Rigotti, M.; Branco, C.S.; Rech, G.; de Oliveira, D.L.; Salvador, M. Grape seed proanthocyanidins improves mitochondrial function and reduces oxidative stress through an increase in sirtuin 3 expression in EA. hy926 cells in high glucose condition. Mol. Biol. Rep. 2020, 47, 3319–3330.
33. Al-Otibi, F.; Alkhudhair, S.K.; Alharbi, R.I.; Al-Askar, A.A.; Aljowaie, R.M.; Al-Shehri, S. The Antimicrobial Activities of Silver Nanoparticles from Aqueous Extract of Grape Seeds against Pathogenic Bacteria and Fungi. Molecules 2021, 26, 6081.
34. Di Stefano, V.; Buzzanca, C.; Melilli, M.G.; Indelicato, S.; Mauro, M.; Vazzana, M.; Arizza, V.; Lucarini, M.; Durazzo, A.; Bongiorno, D. Polyphenol Characterization and Antioxidant Activity of Grape Seeds and Skins from Sicily: A Preliminary Study. Sustainability **2022**, 14, 6702.
35. N. Naseri, H. Valizadeh, P. Zakeri-Milani, Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application, Adv. Pharm. Bull. 5 (3) (2015) 305–313.
36. Li, X.; Qu, J.; Zhang, T.; He, X.; Jiang, Y.; Chen, J. Nuclear Factor kappa B (NF-κB) Targeted Self-Assembled Nanoparticles Loaded with Methotrexate for Treatment of Rheumatoid Arthritis. Med. Sci. Monit. 2019, 25, 8204–8212, doi:10.12659/MSM.917396.
37. Gul, R.; Ahmed, N.; Ullah, N.; Khan, M.I.; Elaissari, A.; Rehman, A.U. Biodegradable Ingredient-Based Emulgel Loaded with Ketoprofen Nanoparticles. AAPS PharmSciTech 2018, 19, 1869–1881, doi:10.1208/s12249-018-0997-0. 88

38. Chantaburanan T, Teeranachaideekul V, Chantasart D, Jintapattanakit A, Junyaprasert VB. Effect of binary solid lipid matrix of wax and triglyceride on lipid crystallinity, drug-lipid interaction and drug release of ibuprofen-loaded solid lipid nanoparticles (SLN) for dermal delivery. J Colloid Interface Sci. 2017;504:247–56.
39. Dolatabadi, S.; Karimi, M.; Nasirizadeh, S.; Hatamipour, M.; Golmohammadzadeh, S.; Jaafari, M.R. Preparation, characterization and in vivo pharmacokinetic evaluation of curcuminoids-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). J. Drug Deliv. Sci. Technol. 2021, 62, 102352.
40. Subramaniam, Z.H. Siddik, N.H. Nagoor, Optimization of nanostructured lipid carriers: understanding the types, designs, and parameters in the process of formulations, J. Nanoparticle Res. 22 (2020) 1–29.
41. Brodusch, N.; Brahimi, S.V.; Barbosa De Melo, E.; Song, J.; Yue, S.; Piché, N.; Gauvin, R. Scanning Electron Microscopy versus Transmission Electron Microscopy for Material Characterization: A Comparative Study on High-Strength Steels. Scanning 2021, 2021, 5511618
42. Almeida H, Lobão P, Frigerio C, Fonseca J, Silva R, Lobo SMS, Amaral MH. Preparation, characterization and biocompatibility studies of thermoresponsive eyedrops based on the combination of nanostructured lipid carriers (NLC) and the polymer Pluronic F- 127 for controlled delivery of ibuprofen. Pharm Dev Technol 2017; 22(3): 336-49.
43. R.A. da Silva-Buzanello, A.C. Ferro, E. Bona, L. Cardozo-Filho, P.H.H. de Araújo, F.V. Leimann, O.H. Gonçalves, Validation of an Ultraviolet–visible (UV–Vis) technique for the quantitative determination of curcumin in poly(l-lactic acid) nanoparticles, Food Chemistry. 172 (2015) 99–104. doi:10.1016/J.FOODCHEM.2014.09.016
44. Hanna D. H.; Saad G. R. Nanocurcumin: Preparation, Characterization and Cytotoxic Effects towards Human Laryngeal Cancer Cells. RSC Adv. 2020; 10: 20724–37.
45. Siregar C. E.; Martono S.; Rohman A. Application of Fourier Transform Infrared (FTIR) Spectroscopy Coupled with Multivariate Calibration for Quantitative Analysis of Curcuminoid in Tablet Dosage Form. J. Appl. Pharm. Sci. 2018; 8: 151–56.
46. Jain, D.; Shivani; Bhojiya, A. A.; Singh, H.; Daima, H. K.; Singh, M.; Mohanty, S. R.; Stephen, B. J.; Singh, A. Microbial Fabrication of Zinc Oxide Nanoparticles and Evaluation of Their Antimicrobial and Photocatalytic Properties. Front. Chem. 2020; 8-14.

47. Banerjee S, Chanda A, Adhikari A, Das AK, Biswas S. Evaluation of phytochemical screening and anti inflammatory activity of leaves and stem of Mikania scandens (l.) wild. Ann Med Health Sci Res 2014; 4: 532-6.
48. Hashemi FS, Farzadnia F, Aghajani A, Ahmadzadeh NobariAzar F, Pezeshki A. Conjugated linoleic acid loaded nanostructured lipid carrier as a potential antioxidant nanocarrier for food applications. Food Sci Nutr. 2020;8:4185–4195.
49. Khosa, S. Reddi, R.N. Saha, Nanostructured lipid carriers for site-specific drug delivery, Biomed. Pharmacother. 103 (2018) 598–613.
50. Beloqui, M.´A. Solinís, A. Rodríguez-Gasc´on, A.J. Almeida, V. Pr´eat, Nanostructured lipid carriers: promising drug delivery systems for future clinics, Nanomedicine 12 (1) (2016) 143–161
51. Gupta, M.; Dey, S.; Marbaniang, D.; Pal, P.; Ray, S.; Mazumder, B. Grape seed extract: Having a potential health benefits. J. Food Sci. Technol. **2019**, 57, 1205–1215. 174.

52. Gomez-Mejía, E.; Roriz, C.L.; Heleno, S.A.; Calhelha, R.; Dias, M.I.; Pinela, J.; Rosales-Conrado, N.; León-González, M.E.; Ferreira, I.C.; Barros, L. Valorisation of black mulberry and grape seeds: Chemical characterization and bioactive potential. Food Chem. **2020**, 337, 127998.
53. Patra, S.; Pradhan, B.; Nayak, R.; Behera, C.; Rout, L.; Jena, M.; Efferth, T.; Bhutia, S.K. Chemotherapeutic efficacy of curcumin and resveratrol against cancer: Chemoprevention, chemoprotection, drug synergism and clinical pharmacokinetics. Semin. Cancer Biol. **2020**, 73, 310–320.
54. S.N. Kanojia N, N. Gupta, S. Singh, Applications of nanostructured lipid carriers: recent advancements and patent review, Biointerface Res. Appl. Chem. 12 (1) (2021) 638–652.
55. Khan I, Hussein S, Houacine C. Fabrication, characterization and optimization of nanostructured lipid carrier formulations using Beclomethasone dipropionate for pulmonary drug delivery via medical nebulizers. Int J Pharm. 2021; 598:120376.
56. Danaei M, Dehghankhold M, Ataei S. Impact of particle size and polydispersity index on the clinical applications of lipidic Nanocarrier systems. Pharmaceutics. 2018;10(2):57.
57. Yoozbashi M, Rashidzadeh H, Kermanian M, Sadighian S, Hosseini MJ, Kaboli Z, et al. Magnetic nanostructured lipid carrier for dual triggered curcumin delivery:

preparation, characterization and toxicity evaluation on isolated rat liver mitochondria. J Biomater Appl. 2022;36(6):1055–63.

58. Sienkiewicz,N.;Członka,S.;Kairyte,A.;Vaitkus,S.Curcuminas a Natural Compound in the Synthesis of Rigid Polyurethane Foams with Enhanced Mechanical Antibacterial and Anti-Ageing Properties. Polym. Test. 2019, 79, 1−29.
59. Salamon, I.; S¸ims¸ek Sezer, E.N.; Kryvtsova, M.; Labun, P. Antiproliferative and Antimicrobial Activity of Anthocyanins from Berry Fruits after Their Isolation and Freeze-Drying. Appl. Sci. 2021, 11, 2096.
60. Chang, C., Meikle, T. G., Su, Y., Wang, X., Dekiwadia, C., Drummond, C. J., et al. (2019). Encapsulation in egg white protein nanoparticles protects anti-oxidant activity of curcumin. Food Chemistry, 280, 65–72.
61. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-inflammatory activity of Ficus racemosa L. bark using albumin denaturation method. AYU 2018;39:239-42.