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Research Article

Formulation and Characterization of Turmeric-Loaded Phytosomes for Targeted Cancer Drug Delivery

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Abstract:

Aims

To develop and evaluate *Curcuma longa* extract phytosomes with enhanced anticancer activity, improved drug solubility, and controlled drug release for potential use in cancer treatment.

Background

Curcuma longa extract has demonstrated potent anticancer activity in vitro. However, its poor solubility limits its therapeutic potential. Phytosome technology can improve the solubility and bioavailability of *Curcuma longa* extract by incorporating it into lipid-based carriers. This study aimed to develop *Curcuma longa* extract phytosomes using lipid, cholesterol, Tween 80, and PEG 400, and to evaluate their physicochemical properties, drug release profile, and anticancer efficacy.

Objective

To formulate *Curcuma longa* extract phytosomes and evaluate their physical characteristics, drug release profile, entrapment efficiency, and anticancer activity against MCF7 cell lines.

Method

Curcuma longa extract phytosomes were prepared using the solvent injection technique followed by high-pressure homogenization to reduce particle size. The formulation was composed of lipid and cholesterol, with Polysorbate 80 (Tween 80) utilized as the surfactant and polyethylene glycol 400 (PEG 400) employed as the co-surfactant to support the formation and stabilization of the system. The optimized formulation was assessed for appearance, pH, stability, particle size, drug release, entrapment efficiency, and in vitro demonstrating anticancer activity against MCF-7 breast cancer cells.

Result

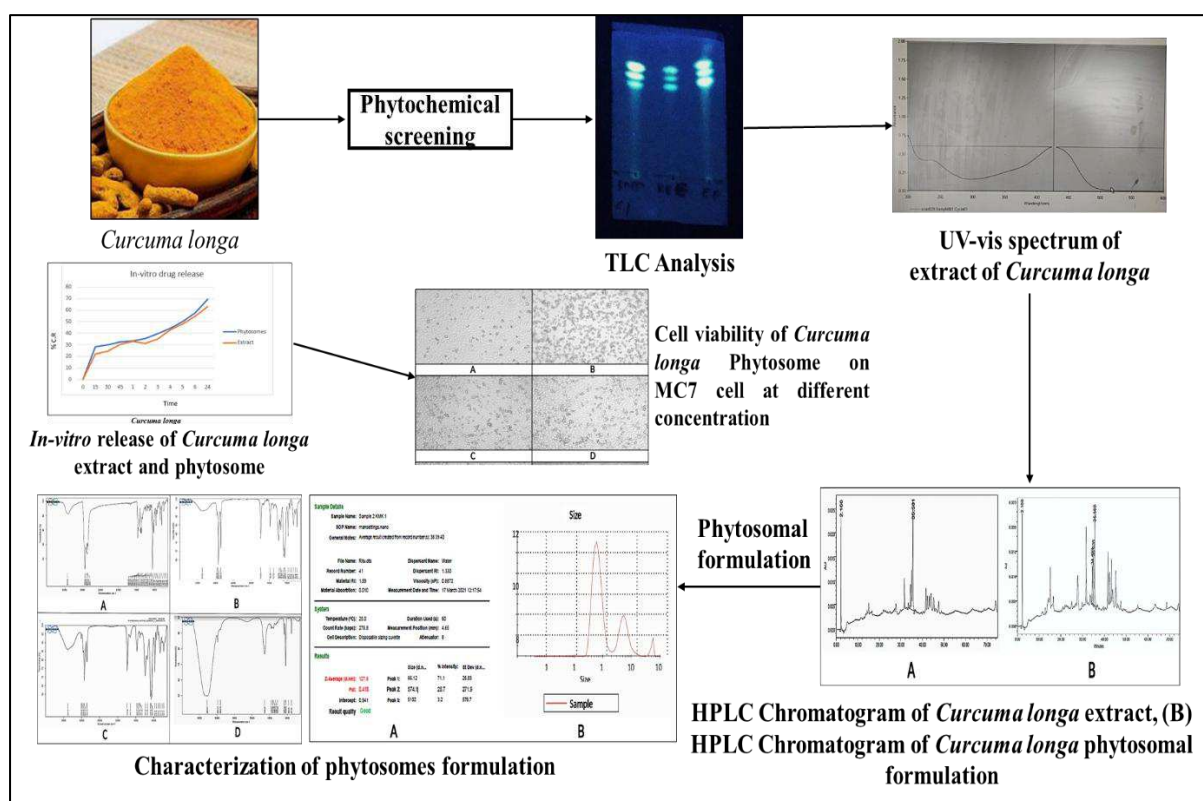
The drug-excipient compatibility study indicated compatibility between components, with a clear formulation and a pH of 7.45. In vitro drug release study revealed 69.3% of drug were released within few hours, with more than 90% released within 24 hours, indicating good controlled-release properties. The entrapment efficiency was 82%, and the optimized batch exhibited an IC₅₀ concentration of 50.19 µg/mL, demonstrating significant anticancer activity.

Conclusion

The developed *Curcuma longa* extract phytosome formulation exhibited enhanced anticancer potential, controlled drug release, and significant activity against MCF7 cell lines. The optimized phytosome formulation presents a promising, safe, and effective approach for cancer treatment.

Keywords: *Curcuma longa*, phytosomes, anticancer, liposomes, MCF7 cell lines

GRAPHICAL ABSTRACT



1. Introduction

Ayurveda uses many herbs as medicines and herb-mineral products to deal with illnesses. The fundamentals underlying the Ayurveda system are true for all times [1–3]. Ayurvedic database contains information about the botanical sources which might be based on past reviews and recent uses [4,5]. This is advantageous to the Drug Discovery process. India possesses a rich heritage of ethnobotanical knowledge dating back to ancient times. [6,7]. *Curcuma longa*, widely recognized as turmeric, has been recognized in Ayurveda for its medicinal properties, particularly in the therapy for a range of diseases, including cancer [8]. The increasing prevalence of cancer, characterized by uncontrolled cell growth and metastasis, has spurred significant interest in finding effective treatments. Conventional cancer therapies, while capable of targeting neoplastic cells, often lead to Significant adverse effect due to their lack of specificity [9]. Research indicates that herbal medicines, particularly those containing active phytoconstituents, can inhibit cancer progression by targeting multiple pathways involved in tumor development [10]. *Curcuma longa* has been highlighted for its potential anticancer properties, with studies demonstrating its ability to suppress tumor initiation, promote apoptosis, and inhibit angiogenesis [11]. The use of phytosomes as enhanced delivery, has emerged as a Promising strategy to enhance the efficacy of herbal extract [12,13]. By encapsulating the active constituents of *Curcuma longa* in phytosomal formulations, it may be possible to enhance their therapeutic effects while minimizing side effects [14]. In the last decade, great emphasis has been placed on identifying natural substances capable of inhibiting, inhibiting, or reversing the multistage carcinogenesis course [15–17]. Herbal medicines and their phytoconstituents have demonstrated anticancer effects by interfering with tumor initiation, progression, angiogenesis, and metastasis [18–20]. Studies suggest that combining herbal therapies with chemotherapy or radiotherapy can enhance overall treatment effectiveness while reducing adverse effects linked to conventional therapies. These agents exert their actions by modulating oncogenes, tumor suppressor genes, epigenetic mechanisms, the tumor microenvironment, and cancer stem cells [21,22]. This research focuses on the formulation and evaluation of turmeric-loaded phytosomes, emphasizing their potential as a safe and effective treatment for cancer. The study aims to develop a stable and effective phytosomal formulation that leverages the therapeutic properties of turmeric to combat cancer cells, particularly in the context of MCF7 breast adenocarcinoma cell lines [14,23,24]. Literature survey claimed *Curcuma longa* as an important plant for treatment of Cancer.

Therefore, *Curcuma longa* phytosome formulation have been used for the treatment of cancer in this research project.

2. Materials

Hydro alcoholic extract of *Curcuma longa*, ethanol, distilled water, Cholesterol, Lipoid P-100 (lipoid Germany), Phosphate buffer 7.4.

2.1 Collection of herbal drugs

Fresh rhizomes of *Curcuma longa* were collected Dr. Palep's Research Laboratory.

2.2 Authentication of *Curcuma longa* rhizome

Authentication of rhizomes of turmeric was carried out at Harshad M. Pandit Laboratory, Mumbai. Its identity confirmed to be *Curcuma longa* L. Family- Zingeberiaceae.

2.3 Extraction of *Curcuma longa*

Soxhlet extraction used for extraction. 10g of turmeric powder was accurately weighed and extracted with ethanol for 24hrs. Extractions were performed in the temperature range of 25-60°C.

2.4 Pre-formulation study

Identify stability, compatibility, solubility, and optimize formulation for drug development.. The primary goals of pre-formulation studies include:

- Identifying the essential physicochemical properties of novel drug substances.
- To determine the kinetics of the release rate.

2.5 Organoleptic Properties

Appearance, colour and odour was determined.

2.6 Solubility Study

Solubility of Turmeric was checked by using different type of organic solvent and water. 10mg of drug was taken in test tubes and recommended volume of solvent was added. After shaking the test tubes clarity of a solution was observed.

2.7 Phytochemical screening of *Curcuma*

Preliminary phytochemical test of ethanolic extract of *Curcuma longa* were performed to identify the presence of phytochemicals using established techniques [25].

2.8 Thin-layer chromatography analysis of the hydroalcoholic (1:1) extract of *Curcuma longa*.

TLC used silica plates with chloroform-acetic acid (9:1) and UV observation at 254/366 nm [26].

2.9 Determination of Maximum Absorption Wavelength of *Curcuma longa*

Curcuma longa extract was prepared, diluted, and analyzed by UV-Vis, showing maximum absorbance at 424 nm.

2.10 Preparation of Standard Calibration Curve for *Curcuma longa*

A standard curve was constructed by measuring absorbance at 424 nm for serial dilutions of *Curcuma longa* extract stock solution.

2.11 HPLC analysis of *Curcuma longa* extract

HPLC method used Acetonitrile-TFA mobile phase, ODS column, 254 nm detection, 1 ml/min flow, 75-minute runtime.

3. Methodology

3.1 Choice of Lipid and Procedure for Phytosome Preparation

The cholesterol and Lipid were selected for phytosome preparation on the basis of their common usage, availability, compatibility with the extract, and their impact on phytosome formation. Several trial batches of phytosomes were prepared using different lipids, and their entrapment efficiency and appearance were evaluated. Among the lipids tested, Lipoid P-100 exhibited superior entrapment efficiency, with no sedimentation observed [27].

3.2 Formulation by solvent injection technique

Lipids (soy phospholipid P100) and Cholesterol were solubilized in 5ml ethanol [alcoholic phase] by ultrasonication. Next, extract of *Curcuma longa* was solubilized in 2ml Hydroalcohol and 4ml phosphate buffer (pH 7.45) by ultrasonication. The solubilized mixture was added to 9ml of phosphate buffer (pH 7.45) and stirred at a low speed of 300rpm [aqueous phase]. After that the alcoholic phase was injected into aqueous phase. After complete addition the speed of the magnetic stirrer was increased to 1200rpm and the stirring was continued for 2hrs solvent evaporated, homogenized vesicular dispersion.. The prepared solution was divided into 2 portions. One portion was kept in the refrigerator and the other portion was kept at room temperature. They were observed for any occurrence of sedimentation. Several batches were prepared using different ratios of excipients based on the trial-and-error method depending on the achievement of maximum entrapment efficiency [15,28].

3.3 Freeze drying of formulation

Freeze-drying (also known as lyophilization) is a widely employed technique for stabilizing therapeutic agents in their dry form. It is used to convert an unstable liquid or suspension into a stable dry product, which can be stored and later reconstituted while retaining the desired characteristics. For the final phytosomal formulation, freeze-drying was performed with 10% w/v D- (+)- Trehalose dihydrate as a cryoprotectant. Cryoprotectants and freeze-drying enhanced phytosome stability, shelf-life, and preserved biological activity [15,16].

3.4 Characterisation of PHYTOSOME formulation

3.4.1 Measurement of particle size

Particle size measurements require highly diluted suspensions to avoid multiple scattering. The optimized phytosomal formulation was diluted with phosphate buffer 7.4 to produce suitable scattering intensity. Particle size measured with Malvern Zeta-sizer after sample dilution and cuvette transfer [18].

3.4.2 Measurement of zeta potential

Zeta potential of optimized phytosomes was measured using Malvern Zetasizer to assess stability, with higher electrostatic repulsion indicating better physical stability of the formulation [18].

3.4.3 Entrapment efficiency (EE)

Entrapment efficiency was calculated by centrifuging phytosomal suspension, measuring free drug concentration, and using a standard calibration curve for *Curcuma longa*. [18]. The drug entrapment efficiency was then computed using the formula:

Entrapment Efficiency (%) = $(\text{Weight of total drug} - \text{Weight of free drug}) \times 100 / \text{Weight of total drug}$

3.4.4 Solid-state characterization

FTIR spectra confirmed solid-state characterization of drug, excipient, and physical mixture.

3.4.5 Stability studies

Phytosome stability assessed following modified ICH guidelines. Phytosome stability was evaluated at 4°C and 25°C for 3 months, assessing drug entrapment and particle size changes.

3.5 *In vitro* release studies

In vitro drug release of Curcuma longa phytosomes was evaluated using the dialysis method in a 250 ml beaker containing 200 ml phosphate buffer (pH 7.4), mimicking physiological conditions. The dialysis membrane (Hi-Media) separated the donor and receptor compartments, and a magnetic stirrer maintained constant agitation at 37°C. A 2 ml sample of phytosomes was sealed in the membrane, which was suspended in the medium at 100 rpm. Samples were collected at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, and 24 hours, filtered, replaced with fresh buffer, and analyzed by UV spectrophotometry at 424 nm.

3.6 Preparation of Phytosomal tablet

3.6.1 Pre-compression parameter of blended powder

3.6.1.1 Angle of repose

The angle of repose was measured using the fixed funnel method, calculating the cone's base diameter to determine stability [19].

$$\theta = \tan^{-1} (h/r)$$

Where:

θ = angle of repose, h = height in cms, r = radius in cms

3.6.1.2 Bulk density

Bulk density measured by transferring powder into a graduated cylinder. Care was taken not to compact the powder, and the volume was noted once the powder settled naturally without tapping. The bulk density, expressed in g/ml, was calculated using the formula:

Bulk density (g/ml) = Mass of the powder / Bulk volume.

3.6.1.3 Tapped density

Tapped density was determined by weighing granules, tapping in a graduated cylinder, and measuring stabilized powder volume after tapping. The tapped density, expressed in g/ml, was calculated using the following equation:

Tapped Density (g/ml) = Mass of Powder / Tapped Volume.

3.6.1.4 Compressibility index

The compressibility index was calculated using Carr's method to assess bulk and tapped density of powder. The formula for calculating Carr's index is as follows:

$$\text{Compressibility Index} = (\text{Tapped Density} - \text{Bulk Density}) / \text{Bulk Density} \times 100$$

3.6.1.5 Hausner's ratio

Hausner's Ratio estimated densification potential using bulk and tapped density formula:

$$\text{Hausner's Ratio} = \text{Tapped Density} / \text{Bulk Density}.$$

3.6.2 Formulation of phytosomal tablet

Curcuma longa phytosomal tablets were prepared by direct compression, blending ingredients, sieving, adding talc, and compressing into 40 mg tablets using flat-faced punches.

3.6.3 Evaluation of PHYTOSOME tablet

3.6.3.1 Weight variation

The weight variation test assesses tablet uniformity by comparing individual tablet weights to the average, ensuring no significant deviations beyond specified limits for compliance [19].

3.6.3.2 Thickness and diameter

The diameter and thickness of the tablet were measured using a Vernier caliper.

3.6.3.3 Hardness test

Tablet hardness was measured using a Monsanto tester, applying pressure until fracture. The force required for fracture, within 4-6 kg/cm², indicates tablet strength.

3.6.3.4 Friability test

Friability was tested using a Roche Friabilator, measuring weight loss after 100 revolutions. The percentage friability should not exceed 1%, calculated by weight difference:

$$\% \text{ Friability} = [(W_1 - W_2) / W_1] \times 100$$

3.6.3.5 Disintegration time

The disintegration test assessed tablet breakdown in 900 ml water at 37°C, with uncoated tablets required to disintegrate within 15 minutes according to pharmacopeial standards [19].

3.6. *In-vitro* dissolution studies

In vitro dissolution of phytosomal tablets was performed using USP Type-II apparatus, analyzing samples at 424 nm and 229 nm after withdrawing 5 mL at intervals [20].

3.7 *In-vitro* cytotoxicity activity of *Curcuma longa* extract phytosome formulation

The samples for anticancer activity were tested in AVERIN BIOTECH PVT. LTD.

3.7.1 Maintenance of cell lines

MCF7 cells were cultured in DMEM with 10% FBS and 1% antibiotic-antimycotic, incubated at 37°C with 5% CO₂, and subcultured every 48 hours [21].

3.7.2 Materials

The experiment utilizes MCF7 human breast adenocarcinoma cells cultured in DMEM-high glucose medium with FBS. Essential reagents include MTT, DMSO, doxorubicin, and D-PBS. Equipment comprises adjustable pipettes, multichannel pipettors, culture plates (96-well) and flasks (T25), centrifuge tubes (50 ml and 1.5 ml), and serological pipettes.

3.7.3 Assay controls

1. **Medium Control:** Contains only the culture medium without any cells, used to account for background absorbance or interference from the medium itself.
2. **Negative Control:** It consists of a culture medium containing viable cells, but without exposure to the test drug or compound, acting as a control to represent normal cell behavior..
3. **Positive Control:** Includes culture medium with cells treated with a known cytotoxic agent—**doxorubicin at 10 µg/ml**—to validate the experimental setup and ensure the assay can detect a cytotoxic effect.

3.7.4 Steps followed

MTT assay evaluated cell viability and IC₅₀ by adding test compound to MCF7 cells, incubating with MTT, dissolving formazan in DMSO, and measuring absorbance at 570 nm for analysis [22,29].

4. Results

4.1 Pre-formulation study

The sample was identified by its appearance, colour, odour and pH which is shown in **Table 1**.

Table 1. Organoleptic Characteristics

Characteristics	Observation
Appearance	Clear solution
Colour	Slightly yellow
Odour	Odourless
pH	7.45

4.2 Solubility

Turmeric extract is soluble in hydro alcoholic solution i.e., Ethanol and water (1:1)

4.3 Phytochemical evaluation of *Curcuma longa*

Phytochemical screening revealed alkaloids, flavonoids, amino acids, saponins, terpenoids.has shown in **Table 2**.

Table 2. Phytochemical tests for identification of chemical constituents in hydroalcoholic extract of the rhizomes of *Curcuma longa* Linn.

Sr. No.	Constituents	Test/Reagent	Observations	Inference
1	Alkaloids	Mayer's test	+	Alkaloid's present
		Dragendroff's test	+	
		Wagner's test	+	
		Hager's test	+	
2	Glycosides	Fehling's test	-	Glycosides absent
		Legal's test	-	
3	Flavonoids	Shinoda test	+	Flavonoid's present
		Zinc chloride reduction test	+	
		Alkaline reagent test	+	
4	Tannins	Gelatin test	-	

				Tannins absent
5	Proteins	Ferric chloride test	-	Proteins absent
		Millon's test	-	
6	Amino acids	Ninhydrin test	+	Amino acids present
7	Fats and fixed oils	Stain test	+	Fats and fixed oils present
		Saponification test	+	
8	Terpenoids	Salkowski test	+	Terpenoids present

4.4 TLC of *Curcuma longa* hydroalcoholic extract

TLC of *Curcuma longa* hydro alcoholic extract is shown in **Figure 1** Mobile phase- Chloroform and Glacial acetic acid (9:1)

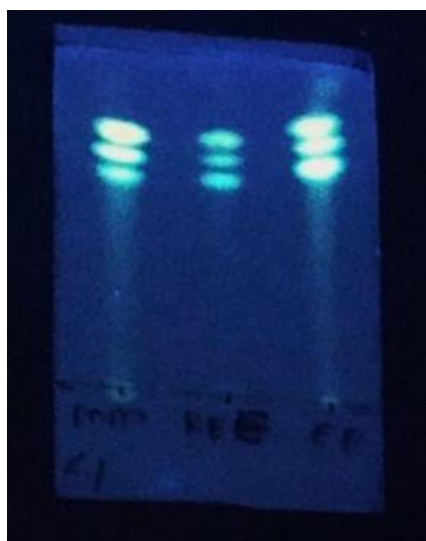


Figure 1. TLC of *Curcuma longa* extract

4.5 Determination of absorption maxima of *Curcuma longa*

UV- scan of hydro alcoholic extract of *Curcuma longa* Linn. was performed from 200-800nm and the $\lambda_{\max}$ (maximum wavelength) was found to be 424nm as shown in **Figure 2, 3, 4 and 5**).

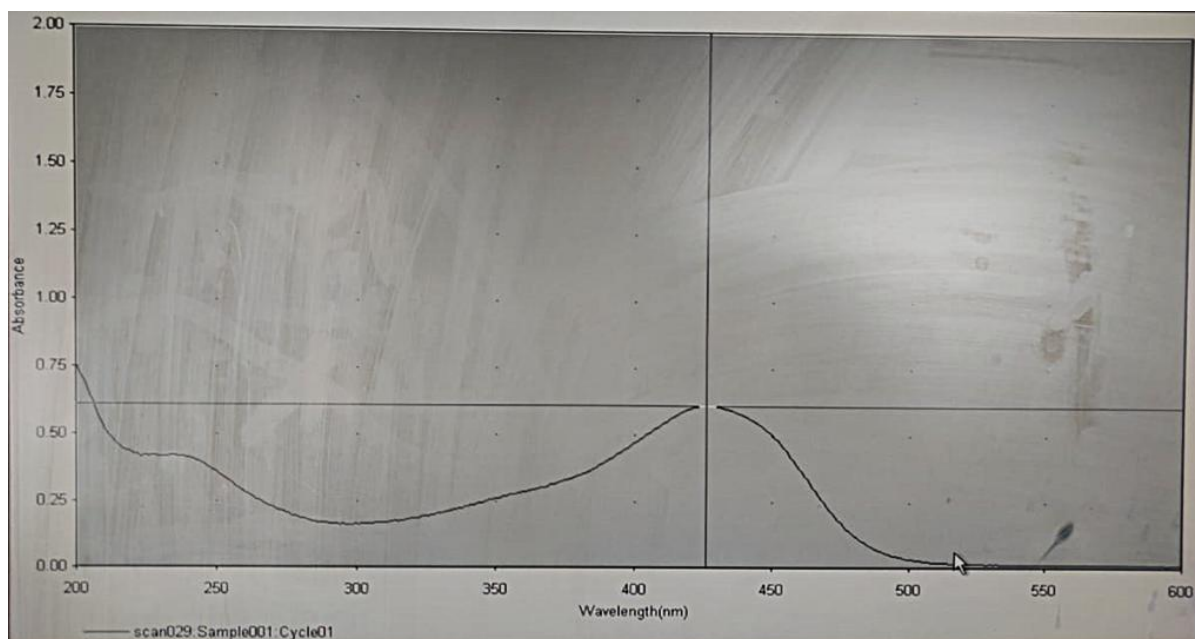


Figure 2. UV-Vis Spectrum Analysis of *Curcuma longa* extract

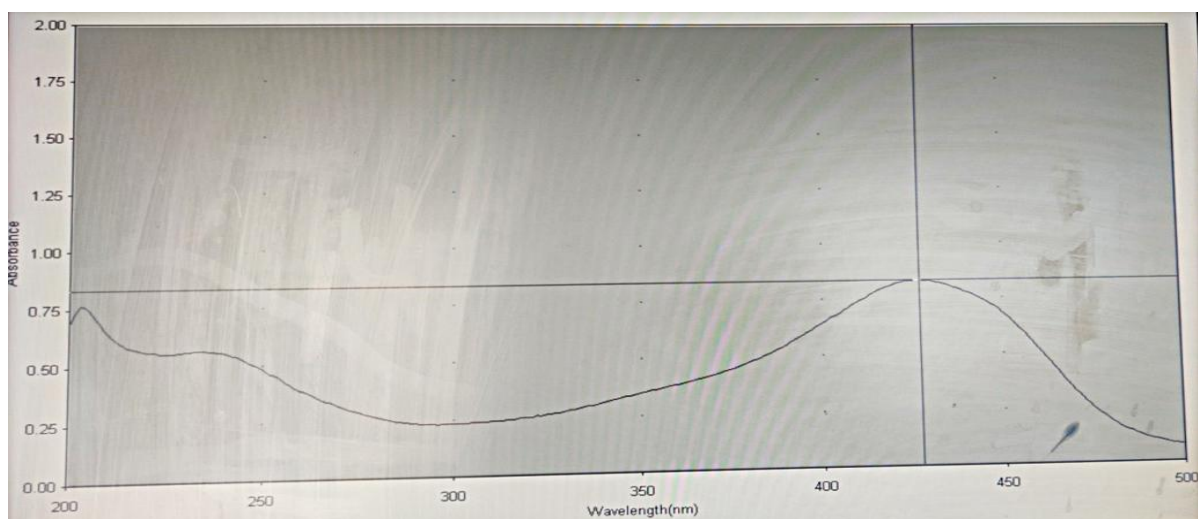


Figure 3. UV-vis spectrum of extract of *Curcuma longa* in phosphate buffer pH 7.45

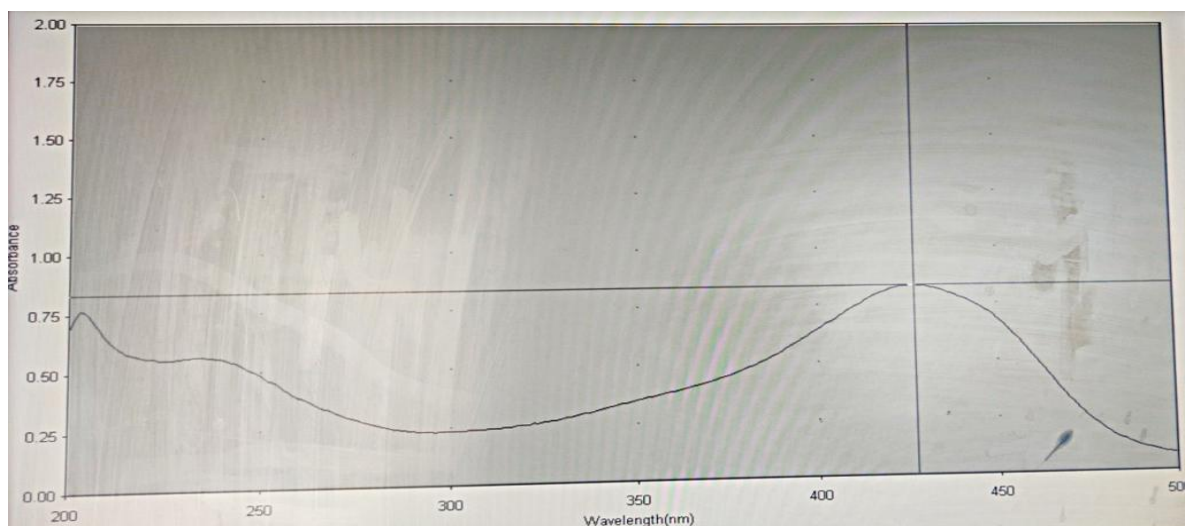


Figure 4. UV-vis spectrum of extract of *Curcuma longa* in 0.1N HCl

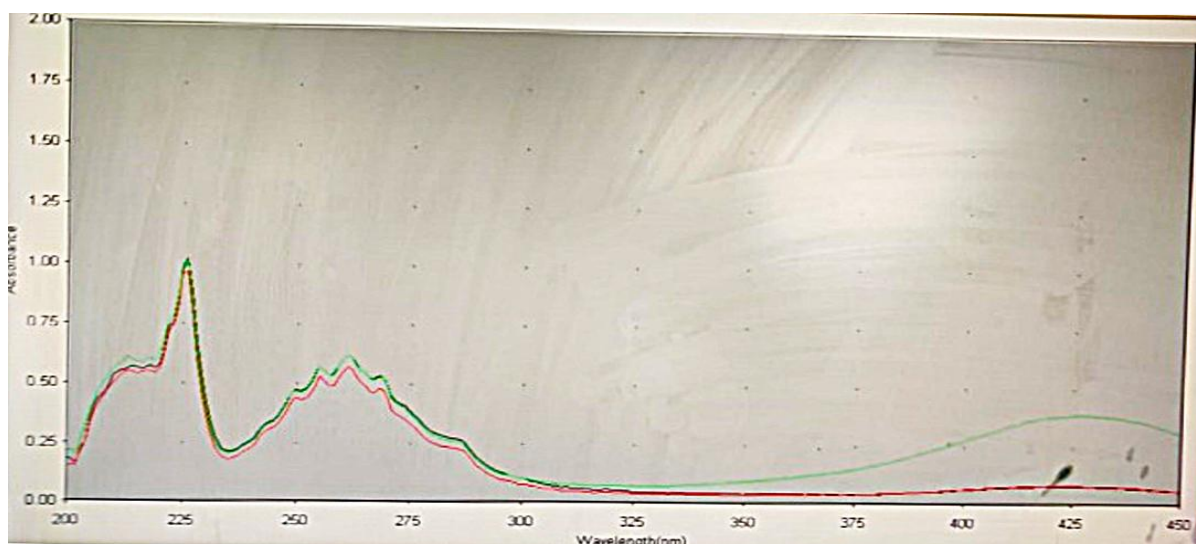


Figure 5. Overlapping peaks of UV-vis spectrum of extract, drug and Standard (Curcumin) of *Curcuma longa*

4.6 Calibration curve of *Curcuma longa* extract

Calibration curve prepared with concentrations 20-180 $\mu\text{g/ml}$; absorbance measured at 424 nm. The calibration curve data and calibration curve of hydro alcoholic extract *Curcuma longa* shown in **Table 3** and **Figure 6**. Calibration curve of *Curcuma longa* in phosphate buffer shown in **Table 4**, **Figure 7**. Calibration curve in 0.1 N HCl shown in **Table 5**, **Figure 8**.

Table 3. Calibration curve data for *Curcuma longa* extract in hydro-alcohol

Sr. No.	Volume withdrawn from stock (ml)	Diluted up to (ml)	Concentration ($\mu\text{g/ml}$)	Absorbance
1.	1	10	20	0.053
2.	2	10	40	0.109
3.	3	10	60	0.173
4.	4	10	80	0.321
5.	5	10	100	0.411
6.	6	10	120	0.485
7.	7	10	140	0.532
8.	8	10	160	0.647
9.	9	10	180	0.743

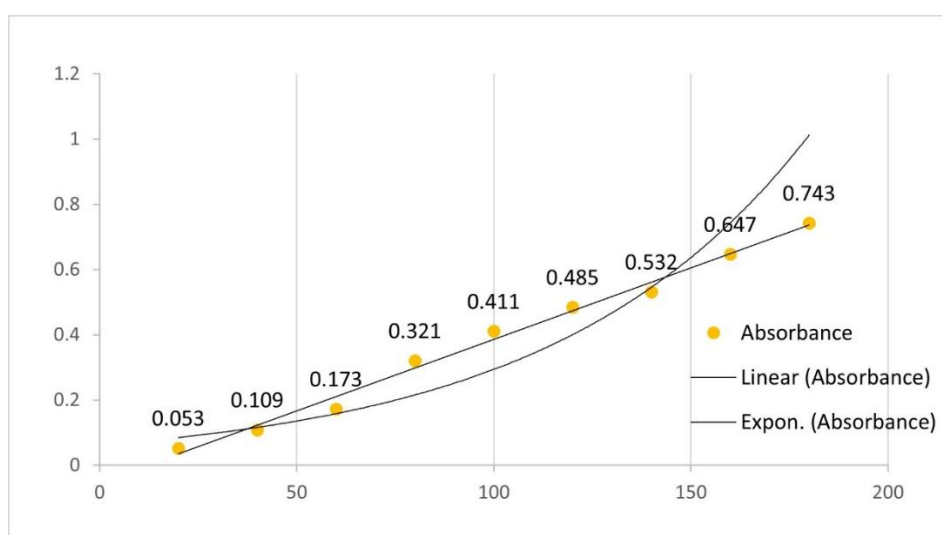
**Figure 6.** Calibration curve of hydro alcoholic extract of *Curcuma longa*

Table 4. Calibration curve data of *Curcuma longa* extract in phosphate buffer pH 7.45

Conc (µg/ml)	Abs
20	0.023
40	0.109
60	0.223
80	0.301
100	0.391
120	0.43
140	0.545
160	0.611
180	0.707

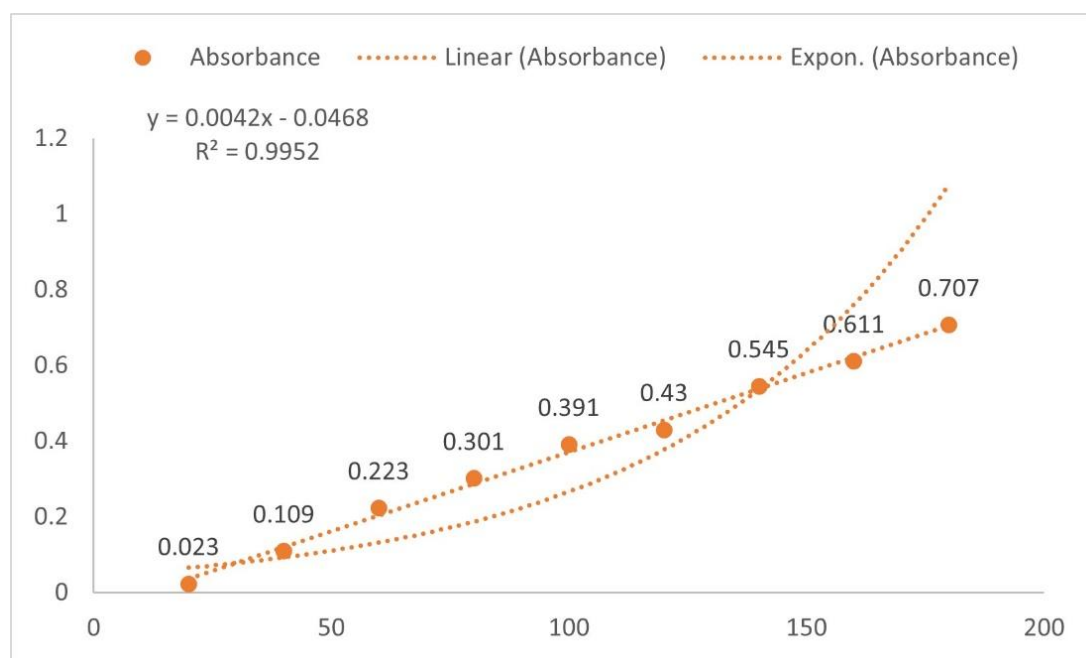
**Figure 7.** *Curcuma longa* extract calibration curve in phosphate buffer pH 7.4.

Table 5. Calibration curve data for *Curcuma longa* extract in 0.1 N HCL

Conc (µg/ml)	Abs
20	0.098
40	0.129
60	0.223
80	0.301
100	0.435
120	0.495
140	0.582
160	0.69
180	0.789

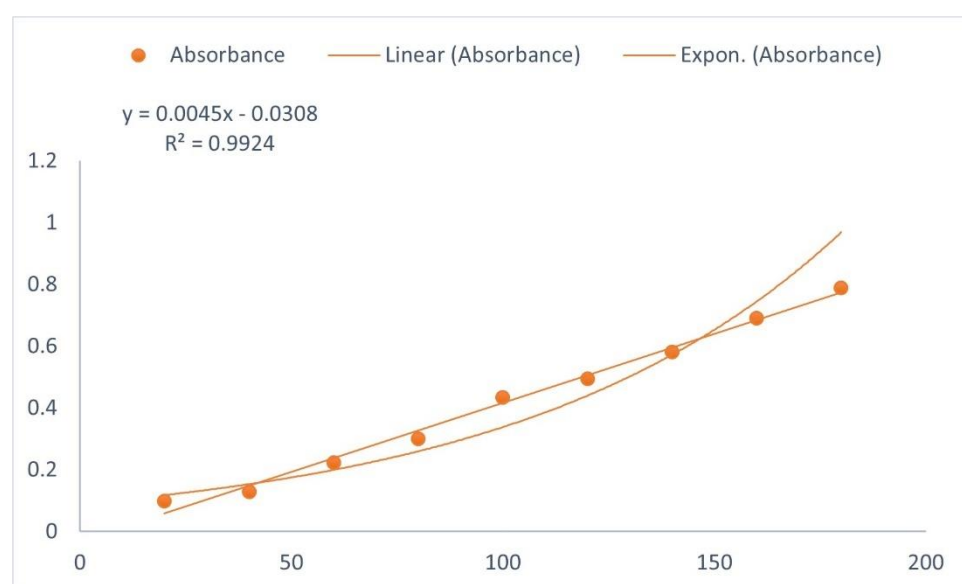


Figure 8. *Curcuma longa* extract calibration curve in 0.1 N HCl.

4.7 HPLC analysis of ethanolic extract of *Curcuma longa*

HPLC Chromatography was carried out at Ultrapure Analytics. HPLC chromatogram of *Curcuma longa* extract and phytosomes shown in **Figure 9**.

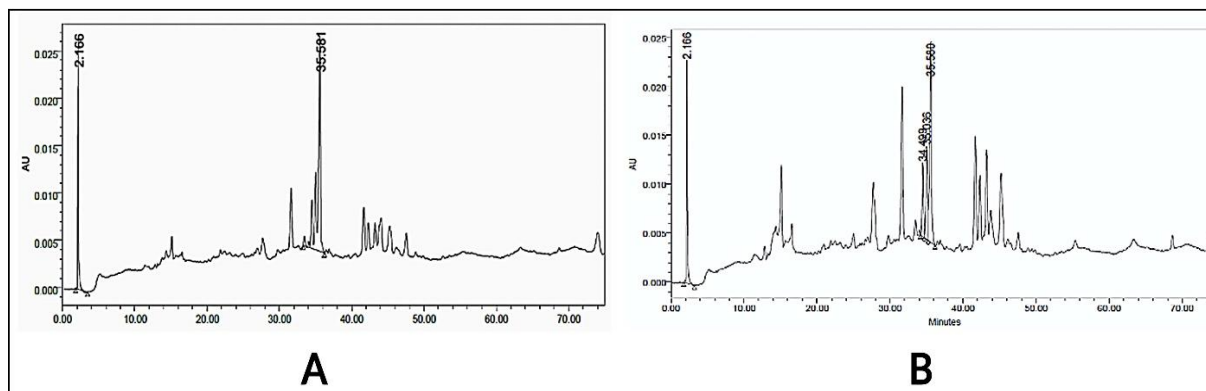


Figure 9. (A) HPLC Chromatogram of *Curcuma longa* extract, (B) HPLC Chromatogram of *Curcuma longa* phytosomal formulation

4.8 Characterization of phytosomes formulation

Particle size analysis of *Curcuma longa* phytosome as shown in **figure 10** (A and B) and Zeta potential analysis of *Curcuma longa* phytosome shown in **figure 11** (A and B).

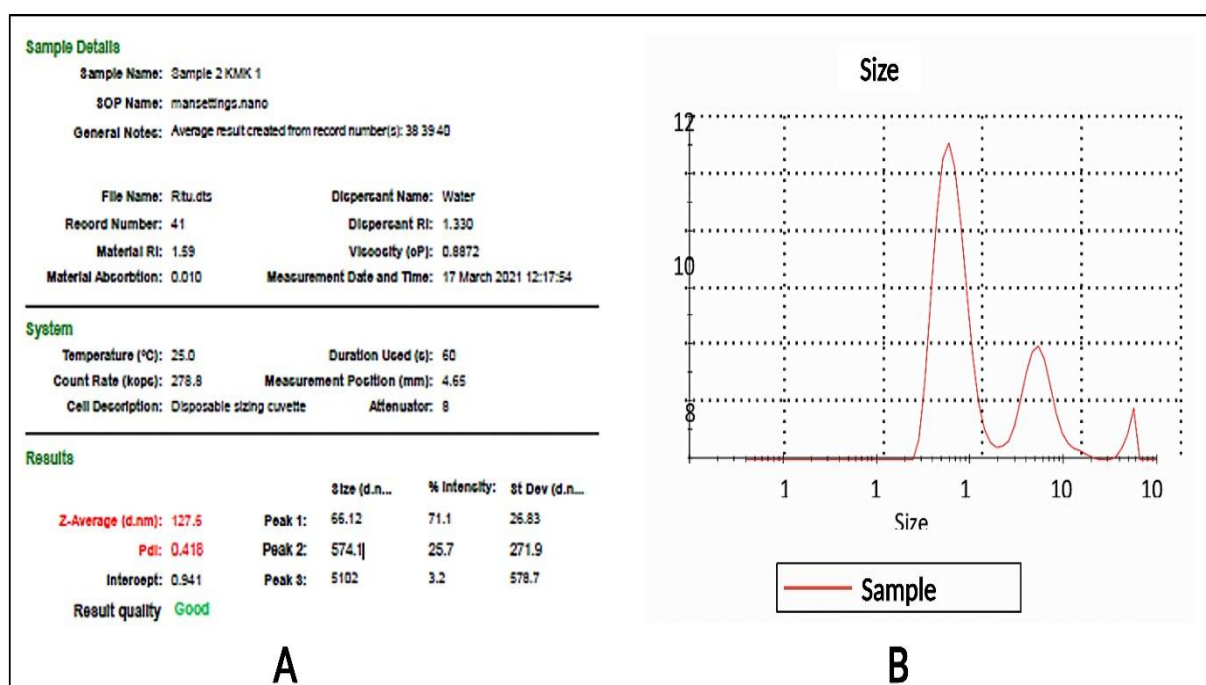


Figure 10. (A) Particle size analysis of *Curcuma longa*, (B) Particle size distribution of *Curcuma longa* phytosomes (F2 formulation)

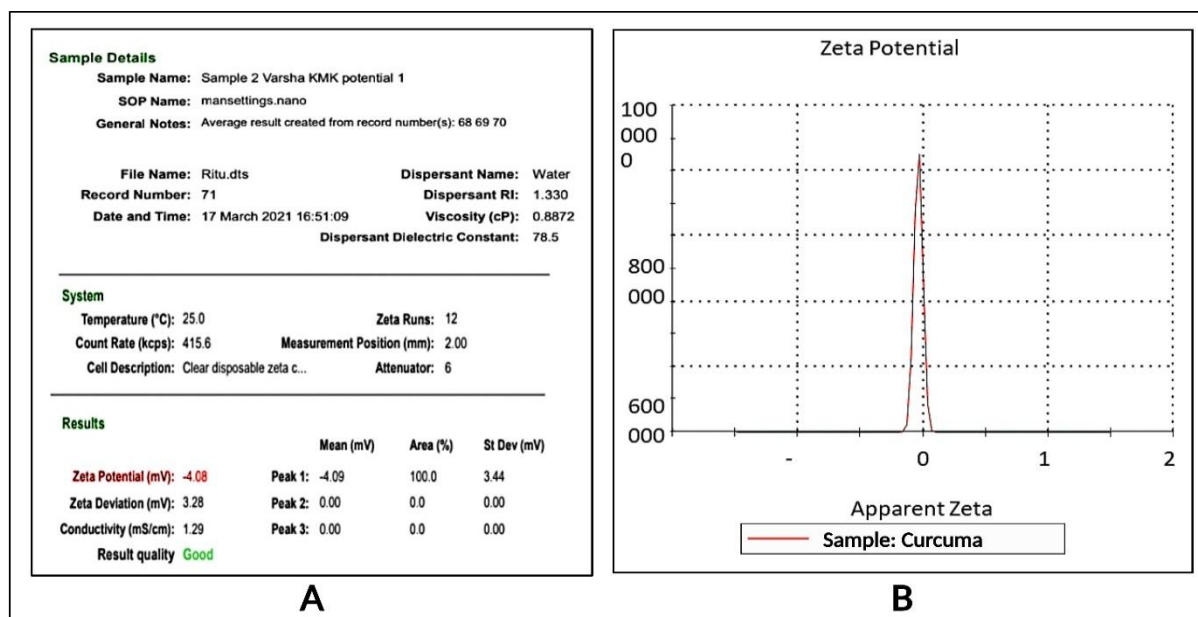


Figure 11. (A) Zeta potential analysis of *Curcuma longa* and (B) Zeta potential of *Curcuma longa* phytosomes (Formulation F2)

4.9 Entrapment efficiency

Entrapment efficiency of *Curcuma longa* Shown in **Table 6**. Maximum entrapment efficiency of *Curcuma longa* phytosomes- 82%.

Table 6. Entrapment efficiency of *Curcuma longa* phytosomes formulation

Formulation code	Entrapment efficiency (%)
F1	56.19
F2	82
F3	34.5
F4	41.73
F5	73.87
F6	29.4

4.10 Solid-state characterization

FTIR Spectra of *Curcuma longa* extract and phytosome shown in **figure 12**.

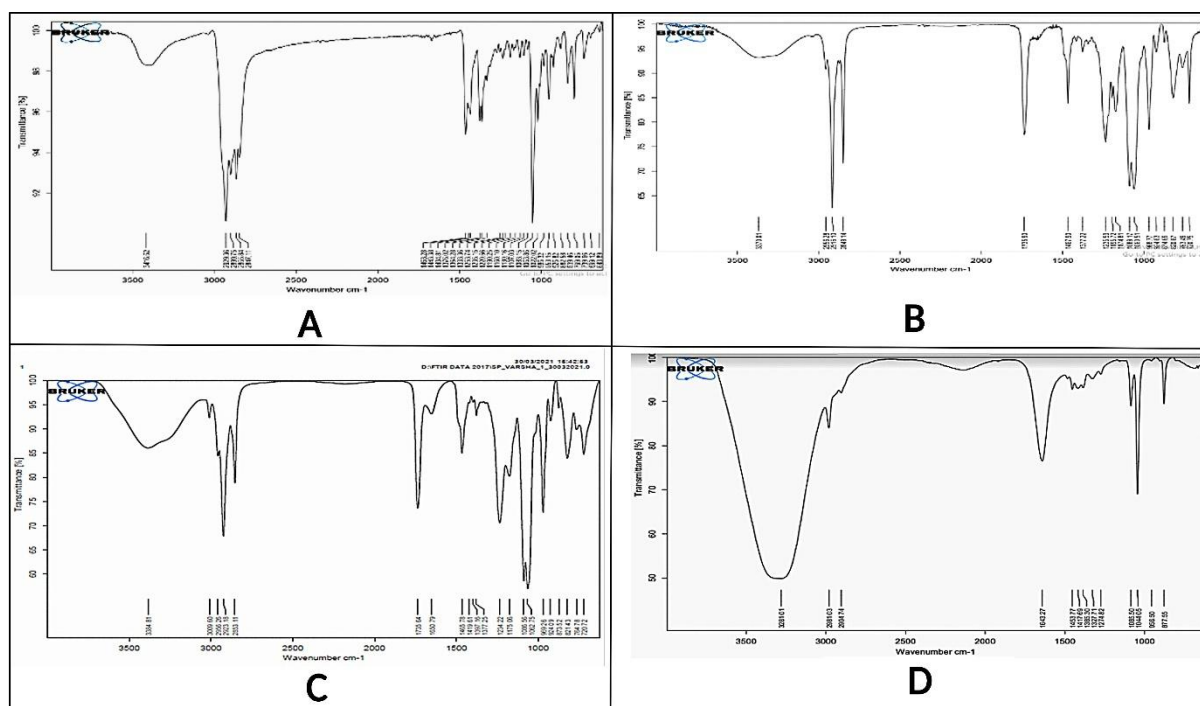


Figure 12. (A) FTIR graph of Cholesterol, (B) FTIR graph of lipid, (C) FTIR graph of *Curcuma longa* extract, lipid and cholesterol, (D) FTIR graph of *Curcuma longa* phytosomes

4.11 Stability Studies

Stability study data of optimized batch of *Curcuma longa* Shown in **Table 7**.

Table 7. Physical stability studies of optimized F2 *Curcuma longa* phytosome.

Sr. No.	Physical stability parameters		Storage temperature	
			4°C	25°C
1.	Clarity test		Pass	Pass
2.	Sedimentation		None	None
3.	Phase separation		None	None
4.	Gelation		None	None
5.	Color change		None	None
6.	Drug entrapment	Initial	82	82%
		3 months	80	79%
		6 months	78	74%

4.11 *In-vitro* release study

In vitro release of *Curcuma longa* phytosome (69.3%) and extract (63.35%) shown in Figure 13.

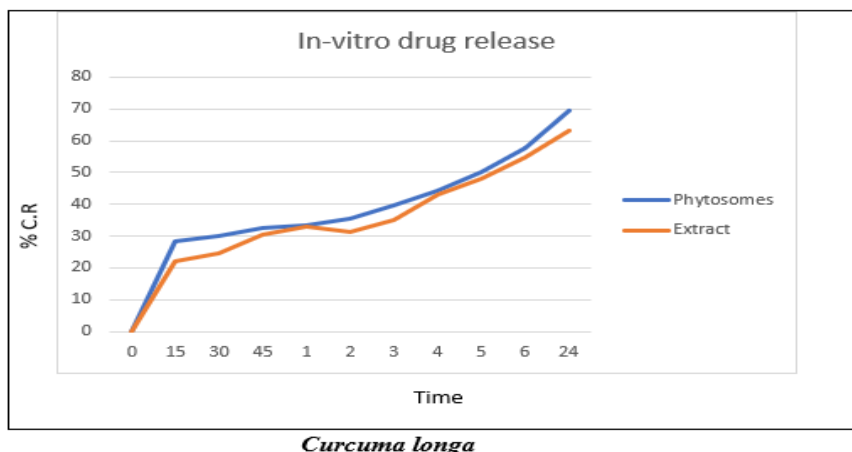


Figure 13. In-vitro release of *Curcuma longa* extract and phytosome.

4.12 Formulation of PHYTOSOMAL tablet

Pre-compression parameter of blended powder shown in **Table 8** and **Table 9**.

Table 8. Pre-compression Parameters of powder for *Curcuma longa* phytosomal tablet

Sr. No.	Parameters	Results	Inference
1	Bulk density (g/cm ³)	0.52	Pass
2	Tapped density(g/cm ³)	0.610	Pass
3	Carr's index (%)	10	Excellent
4	Hausner's ratio	1.13	Poor
5	Angle of repose (°)	23.55	Good

Composition of *Curcuma longa* phytosome shown in table number 9.

Table 9. Composition of *Curcuma longa* phytosomes tablet

Ingredients	Quantity (mg)
<i>Curcuma longa</i> phytosomes	40
Avicel ph 102	135
Anhydrous lactose	70
Aerosil 200	2.5
Talc	2.5
Total	250

4.13 Evaluation of *Curcuma Longa* Phytosomes Tablets

4.13.1 Weight variation

Tablet weights showed no significant variation, within pharmacopoeial acceptable limits.

4.13.2 Thickness and diameter

No significant variation observed in tablet thickness and diameter compared to average.

4.13.3 Hardness test

The hardness of tablets is unacceptable limits i.e., 5Kg/m².

4.13.4 Friability test

All the tablets exhibited a friability of less than 1%, indicating their capacity to resist mechanical stress and shocks that may occur during handling.

4.13.5 In-vitro dissolution study

In vitro diffusion study *Curcuma longa* phytosome & poly-herbs phytosome shown in figure number 14.

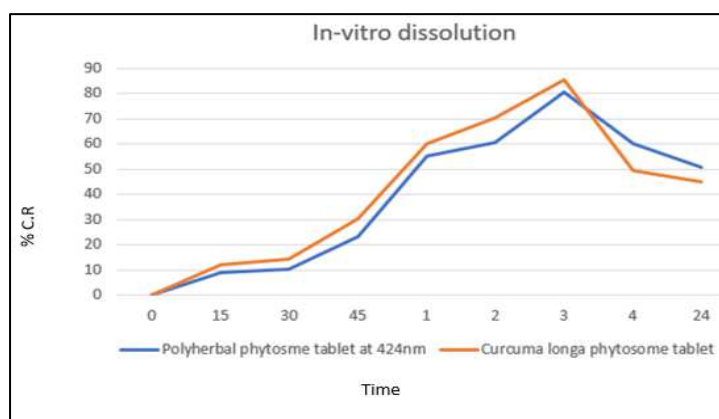


Figure 14. In-vitro diffusion studies of *Curcuma longa* phytosome and polyherbal phytosome tablet

4.13.6 Disintegration test

Indicates that the tablet can withstand physical abrasion. Evaluation parameter of *Curcuma longa* tablet shown in **Table 10**.

Table 10. Physical Characteristics of *Curcuma longa* Phytosomal Tablets

Evaluation parameter	Formulation
Weight variation (mg)	240mg

Diameter (mm)	6
Thickness (mm)	3 ± 0.2
Hardness (kg/cm ²)	4
Friability (%)	0.3 ± 0.12
Disintegration (sec)	320sec

4.14 In-vitro cytotoxic activity

Effect of optimized *Curcuma longa* phytosomal batch on MCF-7 cell lines. MTT assay of *Curcuma longa* Shown in **Table 11**.

Table 11. MTT assay calculations for *Curcuma longa*

Parameter	BLA NK	UNTRE ATED	Standard control	6.25ug/ml	12.5ug/ml	25ug/ml	50ug/ml	100ug/ml
Abs Reading 1	0.043	0.81	0.364	0.764	0.693	0.562	0.449	0.275
Abs Reading 2	0.049	0.815	0.358	0.759	0.686	0.557	0.456	0.28
Mean Abs	0.046	0.8125	0.361	0.7615	0.6895	0.5595	0.4525	0.2775
Mean abs (Sample blank)		0.7665	0.315	0.7155	0.6435	0.5135	0.4065	0.2315
Standard deviation		0.003535	0.00424	0.00353	0.00494	0.00353	0.00494	0.003536

Standard error	0.0025	0.003	0.0025	0.0035	0.0025	0.0035	0.0025
Cell Viability %	100	41.0958	93.3463	83.9530	66.9928	53.0332	30.20
		9041	7965	3327	2453	681	222

Graph and comparative data of cell viability against MCF 7 cell shown in figure number 15 and 16.

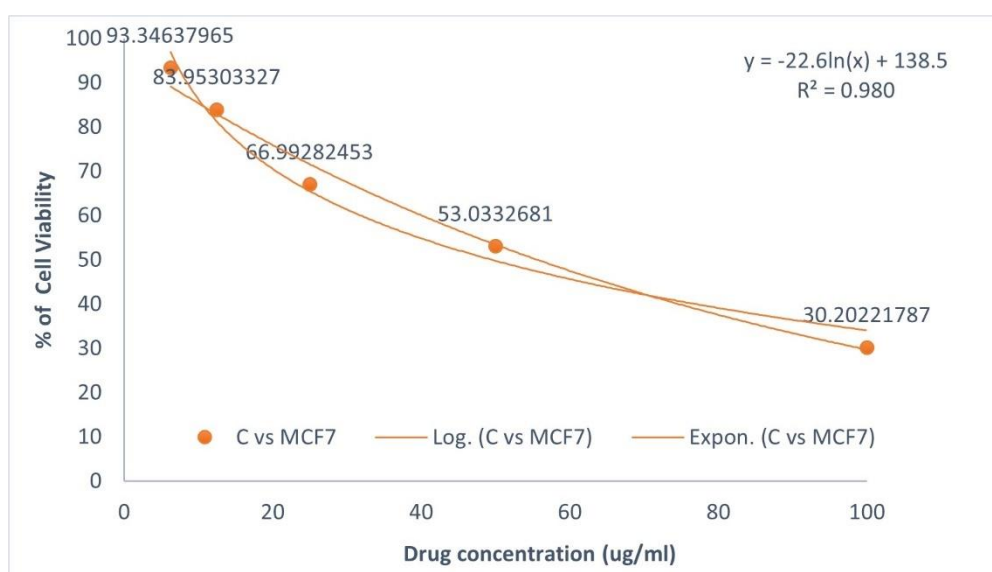


Figure 15. Graph of cell viability vs drug concentration of *Curcuma longa* phytosomes treated against MCF7 cells.

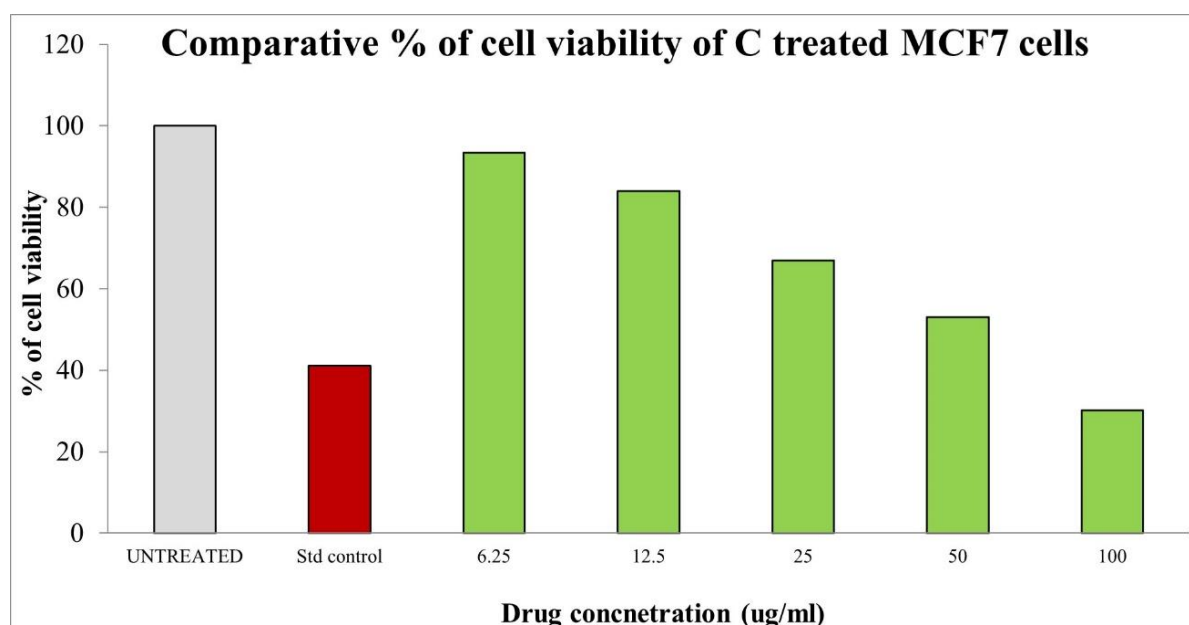


Figure 16. Comparative percentage of cell viability of *Curcuma longa* phytosomes treated against MCF7 cells.

IC 50 concentration of *Curcuma longa* phytosome shown in figure number 17.

Sample: C(*Curcuma longa*)

Test parameter-MTT: IC_{50} (ug/ml) = 50.19

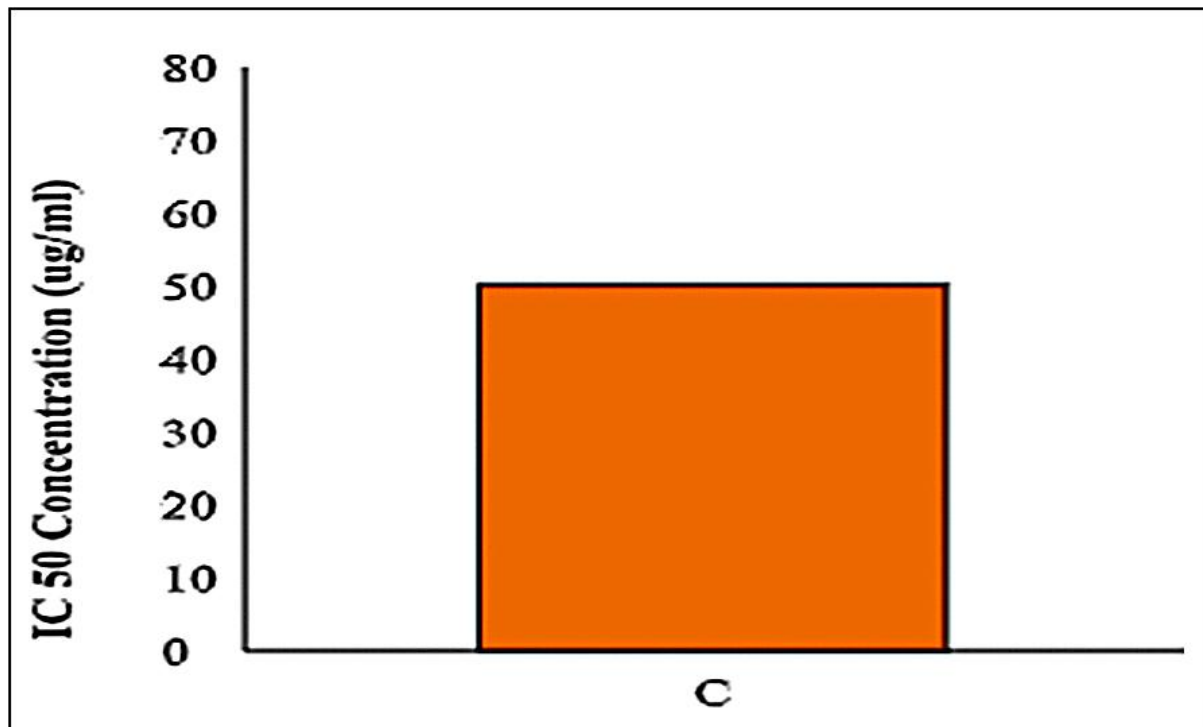


Figure 17. IC 50 concentration of *Curcuma longa* phytosome

Cell viability of *Curcuma longa* Phytosome on MC7 Cell at different concentration shown in figure 18.

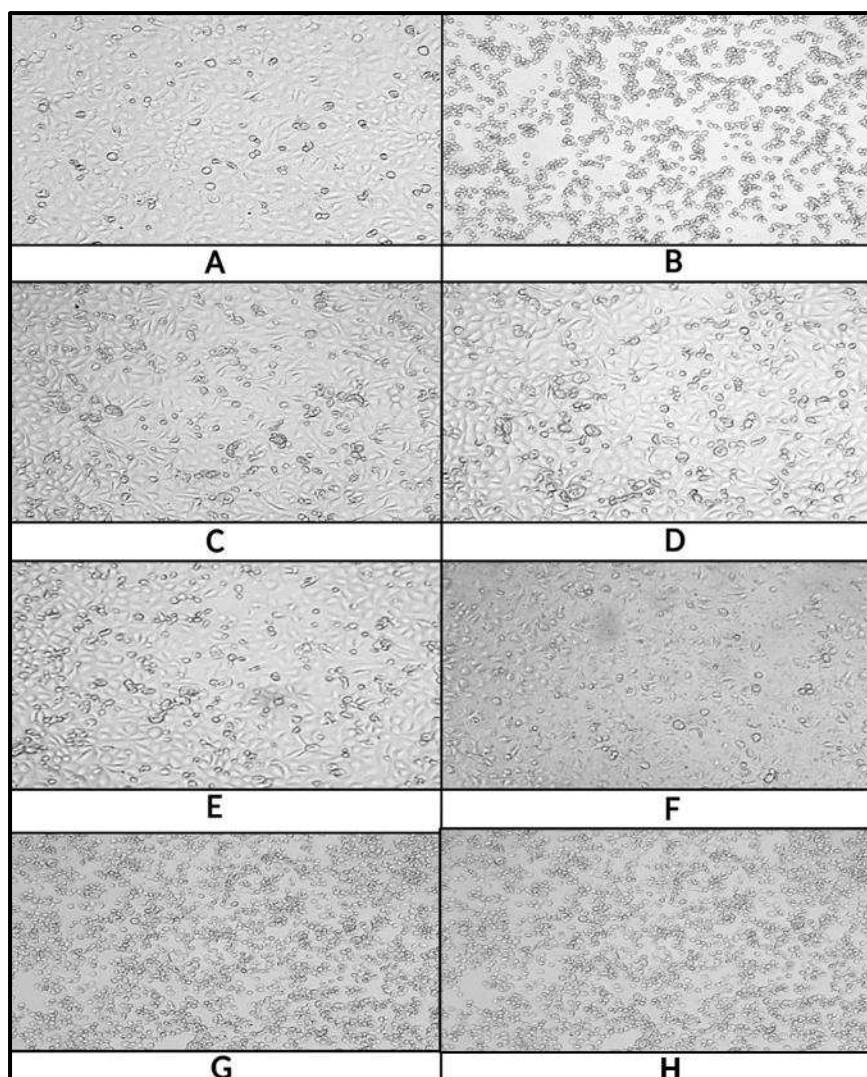


Figure 18. Cell viability of *Curcuma longa* Phytosome on MC7 Cell at different concentration. (A) MCF-7 Untreated, (B) MCF-7 Std control, (C) 6.25uG, (D) 12.5uG, (E) 25uG, (F) 50uG, (G) 100uG.

5. Conclusion

Turmeric is derived from the rhizomes of *Curcuma longa*, a plant belonging to the ginger family (Zingiberaceae). Its hydroalcoholic extract has been shown to inhibit key stages of cancer development, including transformation, tumor initiation, promotion, angiogenesis, cell proliferation, and metastasis. Additionally, it exerts its effects by downregulating various transcription factors, inflammatory cytokines, protein kinases, growth factors, and other oncogenic molecules. *Curcuma longa* phytosomes showed significant cytotoxicity against MCF-7 cells with an IC₅₀ value of 50.19 µg/ml. This low IC₅₀ indicates a strong cytotoxic potential, suggesting that the phytosomal formulation could be a promising anti-breast cancer

agent. Consequently, the phytosome formulation demonstrates both safety and effectiveness as an anti-cancer therapy.

List of Abbreviations

MIC	=	Minimum Inhibitory Concentration
ROS	=	Reactive Oxygen Species
BHT	=	Butylated Hydroxy Toluene
LDL	=	Low Density Lipoprotein
ELISA	=	Enzyme-Linked Immunosorbent Assay
AChE		Acetylcholinesterase

Competing interests

The authors declared that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Availability of data and materials

The data and materials used in this study were collected through original research conducted by the authors.

Author's contributions

This collaborative effort was made possible through the dedicated contributions of all authors. VRG and SSP were involve in the sample collection, extraction, and analysis phases of the study. The manuscript was collaboratively written by VRG, SSP, and ANJ, showcasing a unified vision and scholarly diligence. All authors reviewed the results and approved the final version of the manuscript.

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