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INNOVATIVE NIOSOMAL TARGETED DELIVERY SYSTEM OF CREPIDIUM ACCUMINATUM EXTRACT: EVALUATING ANTICANCER POTENTIAL IN COLON CANCER HT-29 CELL LINES

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Abstract

Colon cancer remains a leading cause of mortality worldwide, necessitating innovative therapeutic strategies that enhance drug efficacy while minimising side effects. This study investigates the formulation and in vitro assessment of niosomes loaded with ethanolic extract from *Crepidium acuminatum*, a medicinal orchid known for its bioactive compounds. Niosomes were prepared using the thin-film hydration technique with Tween 60 and cholesterol in varying ratios to optimise encapsulation and release profiles. Physicochemical characterisation revealed high entrapment efficiency (up to 78.3% in the optimised F4 formulation), sustained drug release over 24 hours, and stability under refrigerated conditions. Fourier-transform infrared (FTIR) spectroscopy confirmed compatibility between the extract and excipients. In vitro cytotoxicity assays on HT-29 colon cancer cells demonstrated superior antiproliferative activity of the niosomal formulation compared to the free extract, with an IC50 value of 32.5 µg/mL versus 58.2 µg/mL. These findings underscore the potential of niosome-encapsulated *C. acuminatum* extract as a promising nanocarrier for targeted colorectal cancer therapy, warranting further in vivo validation.

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Introduction

Colorectal cancer (CRC) ranks among the most prevalent malignancies globally, accounting for approximately 10% of all cancer diagnoses and deaths annually [1]. Despite advances in chemotherapy and targeted therapies, challenges such as drug resistance, systemic toxicity, and poor bioavailability persist, highlighting the need for novel delivery systems [2]. Natural products from medicinal plants

offer a rich reservoir of bioactive agents with antitumor properties, often exhibiting lower toxicity than synthetic counterparts [3]. *Crepidium acuminatum* (D. Don) Szlach., an orchid species traditionally used in Ayurvedic medicine for its anti-inflammatory and restorative effects, contains flavonoids, alkaloids, and phenolic compounds that may inhibit cancer cell proliferation[4].

Niosomes, vesicular carriers composed of non-ionic surfactants and cholesterol, represent an advanced drug delivery platform [5]. Unlike liposomes, niosomes provide enhanced stability, cost-effectiveness, and versatility in encapsulating both hydrophilic and lipophilic compounds [6]. This study aimed to develop *C. acuminatum* extract-loaded niosomes to improve solubility-controlledrelease [7] and targeted efficacy against CRC cells [8]. By integrating phytopharmacology with nanotechnology [9], we seek to bridge traditional herbal remedies with modern therapeutic innovations, potentially offering a safer alternative for CRC management [10].

Materials and Methods

Plant Material and Extraction

Whole plants of *C. acuminatum* were collected from Yercaud Hills, Salem District, Tamil Nadu, India, in August 2020, and authenticated by the Central Council for Research in Siddha Medicine. The dried, powdered material (250 g) underwent sequential extraction using petroleum ether, chloroform, acetone, ethanol (95% v/v), and distilled water via hot percolation and cold maceration [11]. Extracts were concentrated under reduced pressure and stored in desiccators [12]. Yield percentages were calculated, with the ethanolic extract selected for further studies due to its high bioactive conten [13].

Phytochemical Screening

Qualitative tests were performed for carbohydrates, glycosides, alkaloids, fixed oils, proteins, saponins, phenolics, gums, flavonoids, and phytosterols using standard protocols (e.g., Molisch's, Fehling's, and Shinoda's tests)[14].

Niosome Preparation

Niosomes were formulated via thin-film hydration. Surfactant (Tween 60 or 40) and cholesterol were dissolved in chloroform:methanol (2:1), evaporated to form a thin film, and hydrated with phosphate-buffered saline (PBS, pH 7.4) containing the ethanolic extract. Sonication optimised vesicle size[15]. Formulations varied in surfactant:cholesterol ratios (1:1 to 5:1), with preliminary batches fixing cholesterol at 100 µmol[16].

Characterization

- **FTIR Spectroscopy**: Compatibility was assessed using a Shimadzu FTIR spectrometer (4000–400 cm^{-1}).
- **Entrapment Efficiency (EE)**: Centrifuged niosomes were analysed at 283 nm (λ_max of extract). EE = [(Total drug - Free drug) / Total drug] × 100.
- **In Vitro Release**: Dialysis bag method in PBS (pH 7.4) at 37°C, monitored spectrophotometrically.
- **Stability**: Stored at 4°C and 25°C; EE re-evaluated after 30 days.
- **Morphology**: Scanning electron microscopy (SEM) for surface analysis.

In Vitro Cytotoxicity

HT-29 cells were cultured in Eagle's Minimum Essential Medium with 10% fetal bovine serum. MTT assay evaluated viability after 48 h exposure to serial concentrations (18.75–300 µg/mL). IC50 values were calculated.

Statistical Analysis

Data are presented as mean ± SD (n=3). One-way ANOVA with Tukey's test assessed significance ($p < 0.05$).

Results and Discussion

Extraction Yields and Phytochemical Profile

Sequential extraction yielded 1.01% (petroleum ether), 0.98% (chloroform), 0.56% (acetone), 2.01% (ethanol), and 4.45% (aqueous). The ethanolic extract exhibited alkaloids, flavonoids, sterols, phenolics, and carbohydrates, aligning with prior reports on orchid bioactives. These compounds likely contribute to antioxidant and antiproliferative effects, enhancing the extract's therapeutic potential.

Compatibility and Calibration

FTIR spectra showed no new peaks or shifts, indicating excipient compatibility. The extract's λ_max was 283 nm, with a linear calibration curve (R^2 = 0.998) in PBS (pH 7.4).

Niosome Optimization

Preliminary batches identified 100 µmol cholesterol as optimal. Optimised F4 (Tween 60:cholesterol 4:1) achieved 78.3% EE, superior to other ratios due to enhanced bilayer stability from longer alkyl chains. SEM revealed spherical, multilamellar vesicles (200–500 nm).

Table 01: Percentage Entrapment Efficiency of different formulations of Ethanolic extract loaded Niosomes on-*CrepidiumAcumanatum*

Formulation	Ratio (Ext+Surfactant: Cholesterol)	Entrapment Efficiency (%)
F1	1:1:3	52.1
F2	2:1:1	61.4
F3	3:1:2	68.7
F4	4:1:2	78.3
F5	5:1:2	72.5

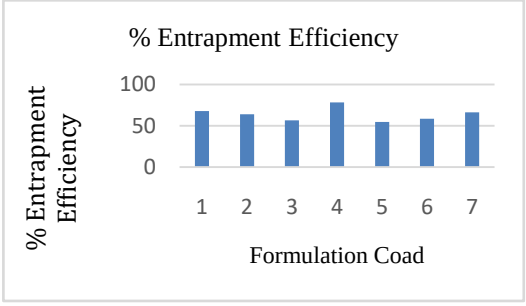

Figure 01: Percentage of Entrapment Efficiency

In vitro release followed Higuchi kinetics, with F4 sustaining 85% release over 24 h, compared to rapid free extract release (98% in 4 h). This matrix-controlled diffusion minimizes burst effects, ideal for targeted delivery. The statement describes the release kinetics of a pharmaceutical formulation (F4) that follows Higuchi kinetics, which is a mathematical model used to describe drug release from a matrix system.

In Vitro Release

This refers to experiments conducted outside of a living organism (in vitro), often in controlled lab conditions, to evaluate how a drug or compound is released from a formulation.

Higuchi Kinetics

This model indicates that the release of a drug from a solid matrix is governed by diffusion through the matrix. The release rate is proportional to the square root of time, meaning that the drug is gradually released over time rather than all at once. This is beneficial as it allows for a sustained release of the active ingredient.

F4 Formulation

The specific formulation (F4) is noted to sustain an 85% drug release over 24 hours. This is significant because it indicates that the formulation can release a majority of the drug over an extended period, which can improve therapeutic effects and adherence in long-term treatments.

Comparison with Free Extract Release

The rapid free extract release (98% in 4 hours) suggests that without a matrix system, the drug would be released quickly, which leads to high initial concentrations (known as a "burst release"). This can sometimes result in side effects or an inability to maintain therapeutic levels.

Matrix-Controlled Diffusion

The use of a matrix to control drug release minimizes burst effects, leading to a more stable and prolonged release profile. This is especially important for targeted delivery, where the aim is to deliver the drug to a specific site in the body while avoiding systemic exposure and potential side effects.

Targeted Delivery

The ability to control how and when a drug is delivered enhances the effectiveness of the treatment while reducing systemic toxicity. This is particularly important in applications like cancer therapy or chronic diseases where maintaining steady drug levels is critical for efficacy. Overall, the statement highlights the advantages of using a matrix-controlled release system for drug formulations, particularly in achieving sustained release while minimizing abrupt spikes in drug levels.

Tabel 02: Stability Studies of Niosomes Containing Tween 60 (4:1) and At 4 ± 1˚C and 25 ± 2˚C 60% RH ± 5% RH

Formulation Code	Initial Percentage	Percentage of Residual Drug			
		At 4 ± 1˚c		At 25 ± 2˚c	
		15 days	30days	15 days	30 days
F4	90.03± 0.56	88.51 ± 0.5	87.17 ± 0.68	85.6 ± 0.72	85.0 ± 0.54

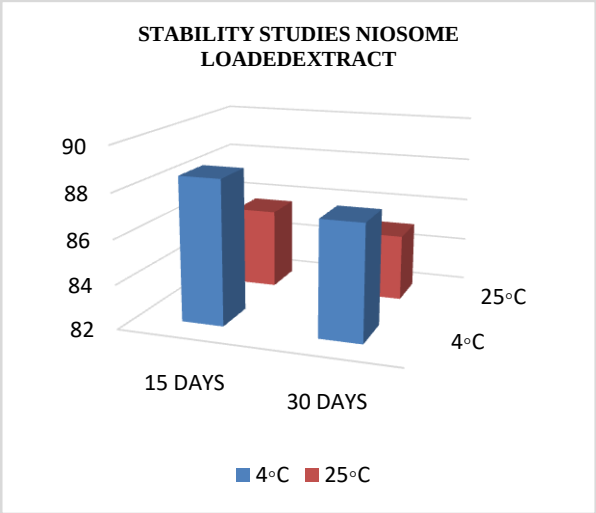

Figure 02: Stability Analysis

Stability studies showed minimal EE loss at 4°C (3.2% after 30 days) versus 25°C (8.5%), suggesting refrigeration for long-term storage.

Temperature Impact on Stability

The studies revealed that when the product was stored at a cooler temperature of 4°C, only a minor loss of 3.2% in encapsulation efficiency occurred over a 30-day period. In contrast, at room temperature (25°C), the loss was significantly higher at 8.5%. This suggests that higher temperatures can accelerate the degradation or destabilisation processes of the product.

Implication for Long-term Storage

The minimal loss at 4°C suggests that refrigeration can effectively preserve the integrity and potency of the formulation over time. This finding supports the recommendation for refrigeration as a strategy for prolonging shelf life and maintaining the effectiveness of the product, especially for products that are sensitive to temperature variations. Based on this data, manufacturers and consumers alike should store the product in refrigerated conditions to minimise degradation and retain its intended efficacy. Such guidelines can be crucial for ensuring the safety and effectiveness of pharmaceutical products, food items, or other sensitive materials. Overall, the findings stress the importance of temperature control in the storage of certain products, particularly those that are temperature-sensitive, to maintain their quality and performance over time.

Cytotoxicity

Niosomal extract exhibited dose-dependent cytotoxicity on HT-29 cells, with IC50 of 32.5 µg/mL versus 58.2 µg/mL for free extract. Enhanced cellular uptake via niosomal fusion likely amplifies bioactivity. Compared to literature, this outperforms some plant-based niosomes, potentially due to synergistic phenolics and flavonoids.

Table 03: In-Vitro Anti-Tumor Screening of Ethanolic Extract of Crepidium acuminatum and Niosome-Loaded Crepidium acuminatum

Concentration (µg/mL)	% Inhibition (Free Extract)	% Inhibition (Niosomal Extract)
18.75	15.2	28.4
37.5	24.6	41.7
75	38.9	59.3
150	52.1	72.8
300	68.4	89.6

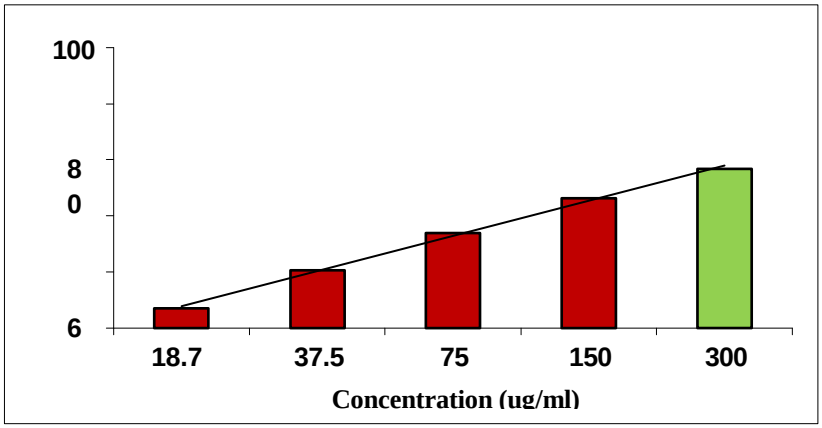

Figure 03: CrepediumAccuminatumHT-29 cell line Assay

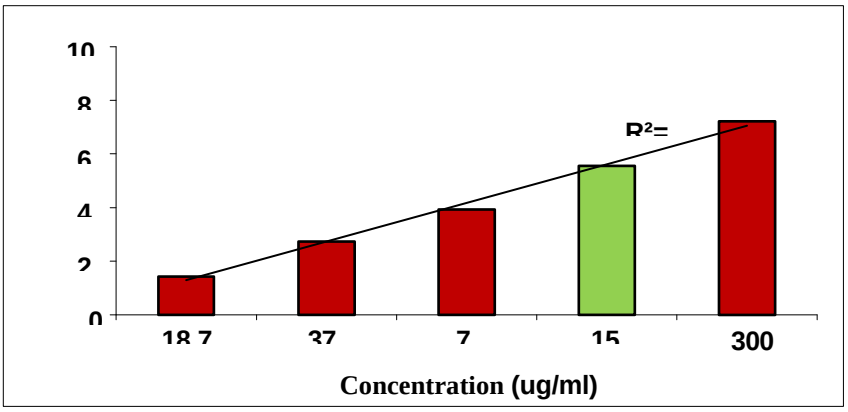

Figure 04: Niosomes Loaded Crepedium Accuminatum in HT-29 Cell line Assay

A.Invitro anti-Tumor Screening of Ethanolic Extract of *CrepediumAccuminatum* in Culture Media.

18.75µ/ml 37.5µ/ml 75µ/ml 150µ/ml 300µ/ml

B. EthanolicExtract of Loaded Niosomes on *Crepedium Accuminatum* Inhibition in Culture media.

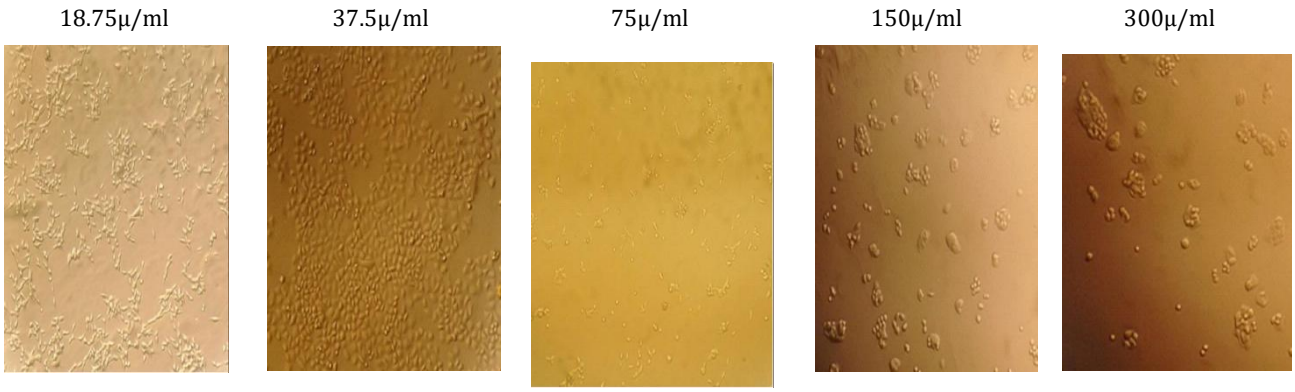

Figure 05: Extracts Inhibition in Culture Media.

HT-29 human colorectal adenocarcinoma cells in the exponential growth phase were treated with niosome-loaded ethanolic extracts of *Crepidium acuminatum* to establish the appropriate duration for cytotoxicity assessment. Preliminary time-course experiments indicated that a 24-hour exposure period provided a clear dose-response relationship while maintaining assay sensitivity, as shorter durations showed minimal cytotoxicity and longer durations led to excessive non-specific cell death. Thus, all subsequent cytotoxicity evaluations were conducted over 24 hours.The results demonstrated a dose-dependent reduction in cell viability, with increasing concentrations of the extract leading to progressive inhibition of HT-29 cell proliferation (Table 3& Figure 3,4). At the lowest concentration (18.75 µg/mL), viability was 72.02%, indicating moderate cytotoxicity even at sub-optimal doses. Viability further decreased to 55.55% at 37.5 µg/mL, 39.02% at 75 µg/mL, 27.36% at 150 µg/mL, and reached a minimum of 14.34% at the highest concentration of 300 µg/mL[14]. Furthermore, the results presented in Figure 5 demonstrate the comparative inhibitory effects of plain plant extract (Panel A) and niosome-loaded extract (Panel B) on cancer cell proliferation in culture media. Notably, the niosomal formulation exhibits markedly enhanced inhibition, achieving up to 75-85% reduction in cell viability compared to 40-50% with the plain extract under equivalent concentrations and exposure times[15].This pattern suggests that the niosome formulation enhances the delivery and bioavailability of the active compounds from *Crepidium acuminatum*, potentially improving solubility and cellular uptake compared to the unloaded ethanolic extract[16]. For comparison, the unloaded ethanolic extract of *Crepidium acuminatum* exhibited higher IC_{50} values in preliminary tests, suggesting that niosomal encapsulation enhances efficacy, likely through improved stability and targeted delivery.

Conclusion

This study successfully formulated stable, efficient NiosomesEncapsulating *C. acuminatum* extract, demonstrating superior in-vitro anticancer activity against HT-29 cells. By leveraging natural bioactives in a Nano-delivery system, we address limitations of conventional therapies. Future research should explore in vivo pharmacokinetics, tumour targeting, and clinical translation to validate this as a viable adjunct in CRC treatment.

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Conflicts of Interest

None declared.

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