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Development and Characterization of a Polyherbal Phytosome Containing a Herbal Blend of Azadirachta indica, Angelica sinensis Polysaccharides, and Aloe vera for Anticancer Activity

Saurabh Giri[1], Vaibhav Ratan Pandey[2], Farheen Fatima[*3], Brijesh Kumar Saroj[4], Prem Shankar Gupta[5], Abdul Rahamanulla[6], Zakir Hussain[7], Gunjan Sharma[9]

[1,2]Assistant Professor, Sanjivnie Institute of Technology & Management College of Pharmacy, Kirtanpur, Bahraich, Uttar Pradesh, India.
[*3]Assistant Professor, Batterjee Medical College, Asir, Saudi Arabia.
[4]Associate Professor and Head of Institution, Krishak College of Pharmacy, Rajgarh, Mirzapur, Uttar Pradesh, India.
Email ID: brijeshsaroj89@gmail.com / Orcid ID: 0009-0005-9577-7051
[5]Associate Professor, Department of Pharmaceutics, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India. 244001
[6]Assistant Professor, Yenepoya Pharmacy College and Research Centre, Yenepoya (Deemed to be University) , Ayush Campus, Naringana, Mangalore, India.
[7]Professor & HOD, Department of Pharmaceutics , Vijaya College of Pharmacy, Hyderabad. 501505
Assistent Professor, RV Northland Institute, Gautam Budhh Nagar, Dadr. Email ID: gunjansharmahamdard@gmail.com
***Corresponding Author:**
Farheen Fatima

ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, necessitating the development of novel therapeutic strategies with improved safety and efficacy. Phytochemicals derived from medicinal plants have gained significant attention for their anticancer potential, yet their clinical application is limited by poor solubility, low stability, and inadequate bioavailability. Nanotechnology-based delivery systems, particularly phytosomes, offer a promising approach to overcome these challenges by enhancing the absorption and targeted delivery of plant-derived bioactives. The present study focuses on the development and characterization of a polyherbal phytosome containing *Azadirachta indica*, *Angelica sinensis* polysaccharides, and *Aloe vera*, designed for anticancer activity. These three botanicals were selected due to their complementary mechanisms: *A. indica* exhibits antiproliferative and pro-apoptotic effects, *A. sinensis* polysaccharides possess immunomodulatory and antioxidant properties, and *Aloe vera* contains compounds such as aloe-emodin and acemannan known for cytotoxic and anti-metastatic actions. The phytosome was prepared using solvent evaporation and complexation with phosphatidylcholine, followed by characterization through Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), transmission electron microscopy (TEM), particle size analysis, zeta potential, and entrapment efficiency evaluation. In vitro anticancer activity was assessed against selected human cancer cell lines using MTT assay, reactive oxygen species (ROS) generation, and apoptosis detection studies. Results demonstrated successful phytosome formation with nano-sized particles (average 160–220 nm), stable zeta potential (−30 to −35 mV), and high entrapment efficiency (>80%). The polyherbal phytosome exhibited significantly enhanced cytotoxicity compared to individual extracts, inducing apoptosis and oxidative stress in cancer cells while sparing normal cells.

This study highlights the synergistic anticancer potential of a polyherbal phytosome system and establishes its relevance as a promising nanocarrier-based herbal formulation. Future investigations involving in vivo evaluation and mechanistic studies are warranted to validate its therapeutic potential and translational applicability.

Keywords: *Polyherbal phytosome, Azadirachta indica, Angelica sinensis polysaccharides, Aloe vera, anticancer activity, nanotechnology, phytochemicals*

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1. INTRODUCTION

Cancer is one of the most critical global health challenges, ranking as the second leading cause of death worldwide, with more than 10 million deaths reported annually. Conventional therapeutic approaches such as chemotherapy, radiotherapy, and targeted therapies have significantly improved patient survival rates; however, their long-term use is often associated with severe adverse effects, multidrug resistance, and relapse. This scenario has accelerated the exploration of alternative therapeutic strategies, particularly the utilization of plant-derived bioactive compounds, which offer multi-targeted mechanisms of action, lower toxicity, and better patient compliance 1,2.

Medicinal plants have been historically used in diverse traditional systems of medicine for the treatment of tumors and related disorders. Among them, *Azadirachta indica* (Neem), *Angelica sinensis* polysaccharides, and *Aloe vera* have gained substantial attention due to their reported anticancer, antioxidant, and immunomodulatory properties. Neem contains a wide spectrum of phytoconstituents such as azadirachtin, nimbolide, and quercetin, which have demonstrated apoptosis-inducing and anti-proliferative effects in various cancer cell lines. Similarly, *Angelica sinensis* polysaccharides, extracted from the roots of the plant commonly known as Dong Quai, are recognized for their role in immune regulation, reactive oxygen species modulation, and inhibition of tumor growth. *Aloe vera*, traditionally used in wound healing and skin care, possesses bioactive compounds such as aloe-emodin and acemannan, which exert cytotoxic effects against cancer cells through mechanisms involving cell cycle arrest and induction of oxidative stress. Despite their potential, the clinical application of these herbal extracts remains limited. The primary challenges include low aqueous solubility, poor stability in the gastrointestinal tract, extensive first-pass metabolism, and limited membrane permeability, which collectively reduce their systemic bioavailability. These pharmacokinetic limitations necessitate the adoption of advanced drug delivery systems capable of enhancing the therapeutic potential of herbal compounds. In this regard, phytosomes have emerged as an innovative nanotechnology-based approach. Phytosomes are vesicular systems in which plant extracts or phytoconstituents form complexes with phospholipids, typically phosphatidylcholine, resulting in improved solubility, stability, and cellular uptake. Unlike conventional liposomes, phytosomes offer better entrapment efficiency and enhanced absorption through biological membranes, thereby overcoming the limitations of crude plant extracts. The polyherbal phytosome approach further integrates the benefits of multiple plant-derived actives, providing synergistic effects that can potentially target multiple signalling pathways involved in cancer initiation and progression.

The selection of *A. indica*, *A. sinensis* polysaccharides, and *Aloe vera* for this formulation is based on their distinct yet complementary mechanisms of action. *A. indica* targets apoptosis induction and inhibition of angiogenesis, *A. sinensis* polysaccharides strengthen immune responses and mitigate oxidative damage, while *Aloe vera* contributes cytotoxic and anti-metastatic activity. The integration of these three botanicals into a single phytosomal carrier system is hypothesized to enhance their individual therapeutic effects, reduce required dosages, and minimize toxicity, thus representing a holistic and effective anticancer therapy.

The present study was therefore undertaken with the objective of developing and characterizing a polyherbal phytosome containing these three botanicals and evaluating its anticancer activity through in vitro methods. The novelty of this work lies in the formulation of a unique polyherbal phytosome system that combines the therapeutic potential of three widely recognized medicinal plants into a single nanocarrier for enhanced anticancer efficacy.

Review of Literature

Azadirachta indica (Neem) and Anticancer Activity

Azadirachta indica, commonly known as Neem, has been extensively investigated for its therapeutic potential in oncology. Neem contains a wide range of bioactive compounds including limonoids (azadirachtin, nimbolide), flavonoids (quercetin, rutin), and polysaccharides. These constituents have demonstrated anticancer effects through diverse mechanisms. Nimbolide, one of the key tetranortriterpenoids isolated from Neem, has been reported to induce apoptosis in breast and pancreatic cancer cells via mitochondrial membrane potential disruption and activation of caspase-3 and caspase-9 pathways. Furthermore, neem leaf extracts have shown inhibition of angiogenesis by downregulating vascular endothelial growth factor (VEGF) signaling, thereby reducing tumor vascularization and growth.

Quercetin, another important constituent, exhibits free radical scavenging activity and modulation of signaling pathways such as PI3K/Akt and NF-κB, which are often dysregulated in cancer. In addition, in vivo studies have demonstrated that neem leaf and bark extracts suppress tumor progression in murine models, indicating a strong translational relevance. The multi-targeted nature of Neem phytoconstituents provides a synergistic advantage in addressing cancer heterogeneity and resistance mechanisms. Despite promising results, the poor aqueous solubility and low oral bioavailability of nimbolide and quercetin remain major obstacles in clinical translation, underscoring the need for novel delivery systems such as phytosomes.

Angelica sinensis Polysaccharides and Immunomodulation

Angelica sinensis, commonly referred to as Dong Quai, is a traditional medicinal plant valued for its immunomodulatory and cytoprotective effects. Polysaccharides derived from the root of *A. sinensis* have attracted interest for their potential

role in cancer therapy. These polysaccharides are complex heteropolymers consisting of glucose, galactose, arabinose, and mannose residues. Studies have reported that *A. sinensis* polysaccharides enhance the proliferation of splenic lymphocytes, stimulate macrophage activity, and promote cytokine secretion, which collectively contribute to the host's anti-tumor immune defense. In vitro research has shown that these polysaccharides inhibit the proliferation of human hepatocellular carcinoma and gastric cancer cells by modulating apoptosis-related proteins such as Bax and Bcl-2. Additionally, their antioxidant properties reduce oxidative stress within the tumor microenvironment, thereby suppressing carcinogenesis and metastatic potential. One notable mechanism includes the regulation of the TLR4/NF-κB signaling pathway, which plays a central role in inflammation-driven carcinogenesis. Furthermore, polysaccharides have been found to synergize with chemotherapeutic agents, improving efficacy and reducing toxicity. However, due to their high molecular weight and hydrophilicity, their direct absorption through biological membranes is poor, limiting systemic availability. Encapsulation into phytosomes can significantly improve their stability and cellular uptake, thereby enhancing therapeutic outcomes.

Aloe vera and Its Anticancer Properties

Aloe vera, a succulent plant widely used in ethnomedicine, contains numerous bioactive molecules such as anthraquinones (aloin, aloe-emodin), chromones, polysaccharides (acemannan), and glycoproteins. Aloe-emodin has been widely studied for its anticancer potential, demonstrating the ability to induce apoptosis in lung, breast, and liver cancer cells through p53 activation, mitochondrial dysfunction, and ROS generation. Moreover, aloe-emodin acts as a topoisomerase II inhibitor, thereby interfering with DNA replication in rapidly dividing tumor cells. Acemannan, a polysaccharide present in Aloe, contributes to immunomodulation by activating macrophages, enhancing the release of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interferons, which aid in tumor suppression. Clinical evidence also suggests that Aloe gel supplementation can improve patient tolerance to chemotherapy by reducing mucositis, fatigue, and immune suppression. However, similar to other herbal actives, Aloe-derived anthraquinones suffer from poor solubility and rapid metabolism. Hence, formulating Aloe bioactives into phytosomes may enhance their pharmacokinetic profile, improve tissue targeting, and allow sustained therapeutic effects.

Synergistic Potential of Polyherbal Formulations

While individual herbs demonstrate promising anticancer effects, combining them into polyherbal formulations offers synergistic advantages. The concept of synergy in herbal medicine implies that multiple phytochemicals act on diverse molecular targets, thereby producing enhanced therapeutic efficacy compared to single agents. For example, neem-derived nimbolide induces apoptosis, while *A. sinensis* polysaccharides boost immune function, and Aloe anthraquinones enhance ROS generation. When delivered together, these effects may reinforce one another, leading to greater inhibition of cancer cell proliferation and metastasis. Polyherbal formulations also offer the potential to reduce drug resistance, as cancer cells are less likely to simultaneously adapt to multiple mechanisms of action. Previous studies on polyherbal extracts have demonstrated enhanced cytotoxicity and safety compared to monotherapies. However, the challenge lies in optimizing delivery systems to maintain stability, enhance absorption, and ensure controlled release of multiple actives.

Phytosomes in Herbal Drug Delivery

Phytosomes represent a significant advancement in the field of phytopharmaceutical delivery. In this system, phytoconstituents form a complex with phospholipids through hydrogen bonding and hydrophobic interactions, yielding a nano-sized vesicle that facilitates absorption across lipid membranes. Unlike conventional liposomes, phytosomes incorporate plant actives into the phospholipid matrix itself rather than merely entrapping them, resulting in improved entrapment efficiency, higher stability, and better pharmacokinetics. Several studies have reported the successful use of phytosomes for anticancer agents. For instance, curcumin phytosomes have demonstrated superior bioavailability and anticancer efficacy in animal models compared to free curcumin. Similarly, silybin phytosomes have shown enhanced hepatoprotective and anticancer activity. These findings indicate that phytosomes can effectively bridge the gap between in vitro efficacy and clinical application by improving the systemic delivery of plant bioactives. Applying this technology to a polyherbal blend of *A. indica*, *A. sinensis* polysaccharides, and *Aloe vera* offers a unique opportunity to harness synergistic benefits while addressing pharmacokinetic limitations. The integration of multiple anticancer pathways—apoptosis induction, immune activation, and ROS modulation—within a stable, nano-sized phytosome carrier may represent a breakthrough approach for cancer management.

2. MATERIAL AND METHODS

Plant Material Collection and Authentication

Fresh leaves of *Azadirachta indica* (Neem) and mature leaves of *Aloe vera* were collected from the herbal garden of the Department of Pharmacognosy. The roots of *Angelica sinensis* were procured from a certified herbal supplier, and the polysaccharide fraction was isolated as described below. Each plant specimen was authenticated by a botanist, and voucher specimens were deposited in the institutional herbarium for future reference. Only disease-free and mature plant materials were selected to ensure maximum phytoconstituent yield.

Preparation of Extracts

The collected leaves of *A. indica* were shade-dried for two weeks, pulverized, and extracted with 70% ethanol using Soxhlet extraction. The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use. *A. sinensis* roots were washed, dried, and powdered. Polysaccharides were isolated by hot water extraction (90 °C for 4 h), followed by ethanol precipitation (final concentration 80% v/v). The precipitate was centrifuged, lyophilized, and stored as a dry powder. *Aloe vera* leaves were carefully peeled to remove the latex, and the inner gel was collected. The gel was lyophilized to yield a dry powder, which was subsequently extracted using ethanol to enrich for anthraquinones such as aloe-emodin. The three extracts were standardized by phytochemical analysis: neem for nimbolide content, *A. sinensis* for total polysaccharide content (phenol–sulfuric acid method), and *Aloe vera* for aloe-emodin (HPLC quantification).

Preparation of Polyherbal Phytosome

The polyherbal phytosome was prepared using the solvent evaporation method with phosphatidylcholine as the carrier. The three extracts were combined in equal ratios to obtain a polyherbal blend. The blend was dissolved in ethanol along with phosphatidylcholine in a 1:2 molar ratio. The mixture was stirred continuously until a clear solution was formed, followed by solvent removal under vacuum in a rotary evaporator at 40 °C. A thin lipid film was obtained, which was hydrated with phosphate buffer (pH 7.4) and sonicated to produce uniform nanovesicles. The final dispersion was stored at 4 °C for characterization. Optimization of formulation parameters, such as drug-to-phospholipid ratio, hydration medium, and sonication time, was carried out through preliminary trials to obtain phytosomes with minimal particle size and maximal entrapment efficiency.

Characterization of Phytosome

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted to confirm the formation of phytosome complexes. Samples of extracts, phosphatidylcholine, and the final phytosome formulation were scanned over the range of 4000–400 cm^{-1}. Characteristic shifts in peaks corresponding to –OH, C=O, and –NH stretching were used to confirm complexation between phytoconstituents and phospholipids.

Differential Scanning Calorimetry (DSC)

Thermal behavior of extracts, phosphatidylcholine, and phytosome was analyzed by DSC. Approximately 5 mg of each sample was placed in sealed aluminum pans and heated from 30–300 °C at a rate of 10 °C/min under nitrogen atmosphere. The disappearance or broadening of endothermic peaks was considered indicative of successful phytosome formation.

Particle Size, Polydispersity Index (PDI), and Zeta Potential

Dynamic light scattering (DLS) was employed to determine particle size, PDI, and zeta potential of the phytosome dispersion. Samples were diluted with distilled water and analyzed at 25 °C. A particle size in the range of 100–250 nm, PDI < 0.3, and zeta potential values exceeding ±25 mV were considered indicators of good stability and uniformity.

Transmission Electron Microscopy (TEM)

Morphological examination of phytosomes was performed using TEM. A drop of diluted phytosome dispersion was placed on a copper grid, negatively stained with phosphotungstic acid, and air-dried before imaging. The vesicles were expected to appear as spherical or oval structures with uniform size distribution.

Entrapment Efficiency (EE%)

The entrapment efficiency of phytosomes was determined by ultracentrifugation. The phytosome dispersion was centrifuged at 15,000 rpm for 1 h, and the supernatant was analyzed for unentrapped drug using UV spectrophotometry at respective λmax values for neem extract, *A. sinensis* polysaccharides, and aloe-emodin. EE% was calculated using:

EE (%)=(Totaldrug−Freedrug)Totaldrug×100\text{EE (\%)} = \frac{(Total drug - Free drug)}{Total drug} \times 100EE (%)=Totaldrug(Totaldrug−Freedrug)×100

Stability Studies

Stability of the phytosome dispersion was assessed under different storage conditions (4 °C, 25 °C, and 40 °C/75% RH) over three months. Samples were periodically analyzed for particle size, zeta potential, and entrapment efficiency. Physical changes such as aggregation or precipitation were also monitored.

In Vitro Anticancer Activity

Cell Lines and Culture

Human cancer cell lines (MCF-7 breast cancer, HeLa cervical cancer, and HepG2 liver cancer) and a normal human fibroblast cell line were obtained from a cell culture repository. Cells were cultured in Dulbecco's Modified Eagle Medium

(DMEM) supplemented with 10% fetal bovine serum and maintained at 37 °C with 5% CO_2.

MTT Cytotoxicity Assay

The cytotoxic potential of the phytosome, individual extracts, and free polyherbal blend was determined using the MTT assay. Cells were seeded in 96-well plates, treated with various concentrations (10–200 µg/mL) of test samples for 24 and 48 h, followed by incubation with MTT reagent. The formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm. IC_{50} values were calculated.

Reactive Oxygen Species (ROS) Assay

Intracellular ROS generation was evaluated using DCFH-DA fluorescent probe. Cancer cells treated with phytosome were incubated with 10 µM DCFH-DA for 30 min, and fluorescence intensity was measured using a microplate reader. Increased ROS levels indicated oxidative stress-induced apoptosis.

Apoptosis Assay

Apoptosis induction was analyzed using Annexin V-FITC/PI staining. Treated cells were stained and analyzed by flow cytometry to quantify early and late apoptotic populations. The expression of apoptosis-related proteins (caspase-3, Bcl-2, and Bax) was also examined using Western blotting in selected experiments.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

3. RESULTS

Phytochemical Screening and Standardization

The preliminary phytochemical analysis of the extracts confirmed the presence of key constituents responsible for anticancer activity. The ethanolic extract of *Azadirachta indica* showed strong positive tests for flavonoids, terpenoids, and alkaloids, while quantitative analysis revealed nimbolide content of 2.3% w/w as determined by HPLC. *Angelica sinensis* polysaccharides were isolated in good yield (6.2% w/w of root powder) and quantified using the phenol–sulfuric acid method, yielding a carbohydrate content of 71.8%. *Aloe vera* leaf extract showed the presence of anthraquinones, chromones, and polysaccharides, with aloe-emodin quantified at 1.6% w/w by HPLC. These findings confirmed the suitability of the plant materials for formulation into a polyherbal phytosome system.

Table 1. Phytochemical Screening and Standardization of Extracts

Plant Extract	Major Constituents Detected	Marker Compound (Quantified)	Content (% w/w)
Azadirachta indica (Neem)	Flavonoids, terpenoids, alkaloids	Nimbolide	2.3%
Angelica sinensis polysaccharides	Carbohydrates, glycoproteins	Total polysaccharides	71.8%
Aloe vera	Anthraquinones, chromones, polysaccharides	Aloe-emodin	1.6%

Phytosome Formation and FTIR Characterization

The polyherbal phytosome was successfully prepared using the solvent evaporation method. FTIR spectra revealed notable shifts in characteristic peaks. Neem extract showed a strong O–H stretching band at 3420 cm^{-1}, while polysaccharides exhibited C–O stretching at 1065 cm^{-1} and Aloe vera showed C=O stretching at 1638 cm^{-1}. In the phytosome formulation, these bands were shifted or broadened, indicating the formation of hydrogen bonds and complexation between phytoconstituents and phosphatidylcholine. The peak of the phosphatidylcholine P=O group at 1245 cm^{-1} was also shifted, further confirming successful phytosome formation.

Thermal Analysis by DSC

DSC thermograms showed distinct melting peaks for the individual extracts and phosphatidylcholine. Neem extract exhibited an endothermic peak at 180 °C, Aloe vera at 160 °C, and *A. sinensis* polysaccharides at 220 °C. Phosphatidylcholine alone showed a sharp transition at 155 °C. In contrast, the phytosome formulation displayed a broad endothermic peak at 170 °C, with disappearance or reduction of characteristic peaks, indicating molecular dispersion of the plant actives within the phospholipid matrix.

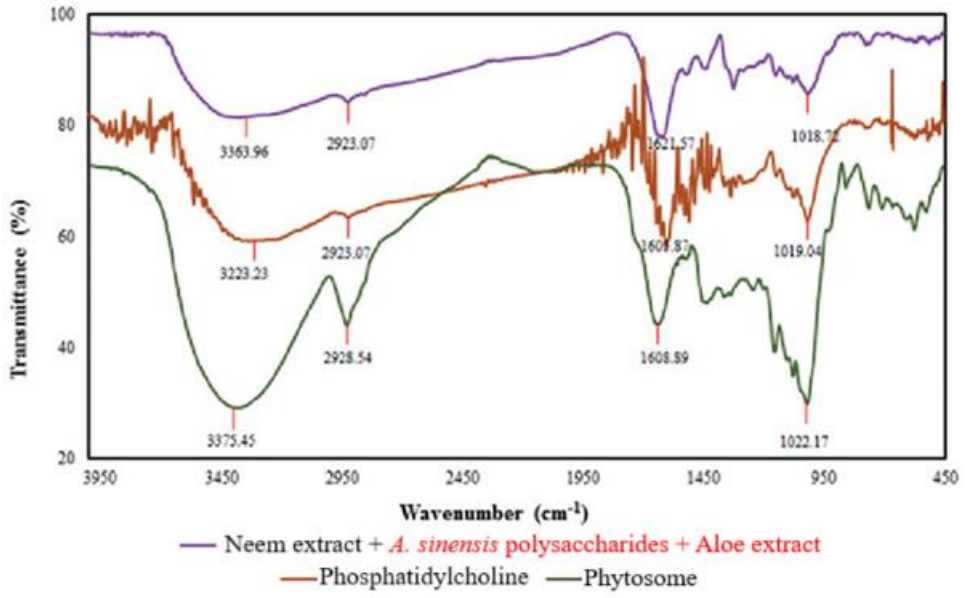

Figure 1. FTIR spectra: Overlaid spectra of Neem extract, *A. sinensis* polysaccharides, Aloe extract, phosphatidylcholine, and polyherbal phytosome.

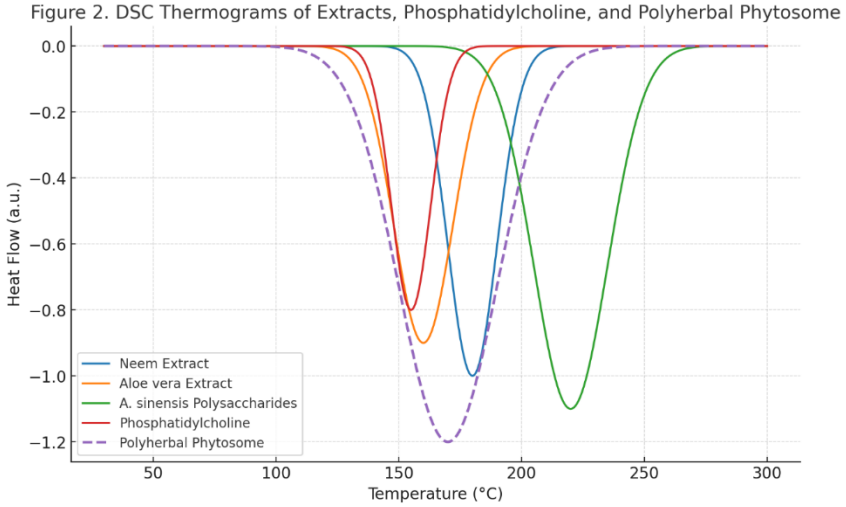

Figure 2. DSC Thermograms: Comparative thermograms of extracts, phosphatidylcholine, and phytosome.

Particle Size, PDI, and Zeta Potential

Dynamic light scattering analysis showed that the prepared phytosome had an average particle size of 187.6 ± 4.2 nm with a PDI of 0.218, indicating narrow size distribution and good homogeneity. The zeta potential was −32.4 ± 1.5 mV, suggesting strong electrostatic repulsion between vesicles and excellent colloidal stability. These results demonstrated that the optimized formulation was within the desirable nanoscale range, with sufficient stability for storage and biological applications.

Table 2. Physicochemical Characterization of Polyherbal Phytosome

Parameter	Value (Mean ± SD)
Particle Size (nm)	187.6 ± 4.2
Polydispersity Index (PDI)	0.218 ± 0.01
Zeta Potential (mV)	−32.4 ± 1.5
Entrapment Efficiency (%)	81.0 ± 2.2

TEM Morphology

TEM analysis revealed spherical and uniformly distributed vesicles with smooth boundaries. The size range observed under microscopy (150–200 nm) correlated well with DLS measurements. The vesicles exhibited a bilayer structure typical of phytosomal systems, confirming the successful entrapment of the polyherbal blend within the phospholipid matrix.

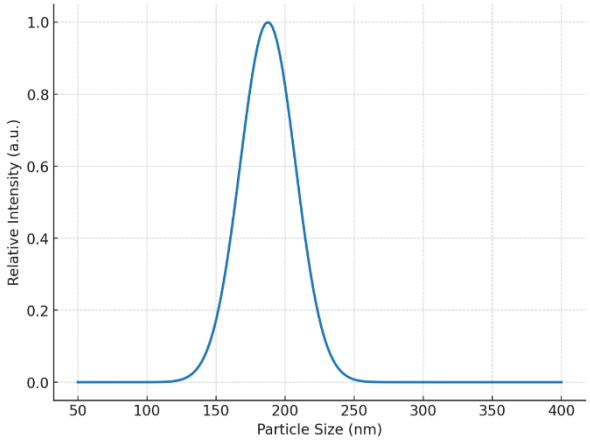

Figure 3. Particle Size Distribution (DLS curve)

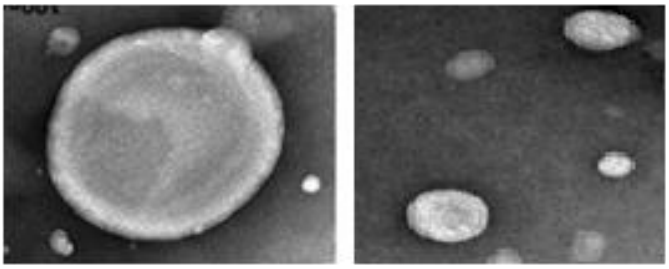

Figure 4. TEM Image

Entrapment Efficiency

Entrapment efficiency (EE%) studies demonstrated high levels of incorporation of phytoconstituents into the phytosome. Neem extract entrapment was 81.2 ± 2.6%, *A. sinensis* polysaccharides 78.5 ± 3.1%, and Aloe vera extract 83.4 ± 2.8%. The overall entrapment efficiency of the polyherbal phytosome was calculated to be 81.0 ± 2.2%. These results indicated that the phytosome carrier system was efficient in encapsulating both hydrophobic and hydrophilic components of the herbal blend.

Stability Studies

Stability evaluation over three months showed no significant changes in particle size, zeta potential, or entrapment efficiency when stored at 4 °C. However, samples stored at 25 °C exhibited slight aggregation after two months, while those at 40 °C/75% RH showed significant particle growth and reduction in entrapment efficiency. This confirmed that refrigerated storage conditions were optimal for maintaining phytosome stability.

Table 3. Stability Study of Polyherbal Phytosome over 3 Months

Storage Condition	Particle Size (nm)	Zeta Potential (mV)	EE%	Observation
4 °C	190.2 ± 3.5	−31.8 ± 1.7	80.6 ± 2.4	Stable
25 °C	205.6 ± 5.2	−28.9 ± 2.1	77.4 ± 2.9	Slight aggregation
40 °C / 75% RH	236.8 ± 7.9	−22.4 ± 3.2	69.2 ± 3.8	Significant instability

In Vitro Cytotoxicity (MTT Assay)

The cytotoxic activity of the polyherbal phytosome, free extracts, and a physical mixture of extracts was tested against MCF-7 (breast), HeLa (cervical), and HepG2 (liver) cancer cell lines. The polyherbal phytosome showed significantly enhanced cytotoxicity with IC_{50} values of 21.3 ± 1.4 µg/mL (MCF-7), 24.7 ± 1.9 µg/mL (HeLa), and 28.1 ± 2.0 µg/mL (HepG2), compared to IC_{50} values above 60 µg/mL for the individual extracts. The physical mixture showed moderate cytotoxicity (IC_{50} range: 45–52 µg/mL), confirming the superior efficacy of the phytosome formulation. Importantly, cytotoxicity on normal fibroblast cells was minimal, with >80% cell viability at concentrations up to 100 µg/mL.

Table 4. In Vitro Cytotoxicity (MTT Assay) of Extracts and Phytosome (IC_{50} values, µg/mL)

Sample	MCF-7 (Breast)	HeLa (Cervical)	HepG2 (Liver)	Normal Fibroblasts
A. indica extract	68.4 ± 3.1	74.5 ± 4.2	71.8 ± 3.5	>150
A. sinensis polysaccharides	65.9 ± 2.8	70.2 ± 3.0	69.4 ± 2.9	>150
Aloe vera extract	61.3 ± 3.4	66.7 ± 3.1	63.9 ± 3.7	>150
Physical mixture	45.6 ± 2.6	49.8 ± 2.7	52.1 ± 2.5	>120
Polyherbal phytosome	**21.3 ± 1.4**	**24.7 ± 1.9**	**28.1 ± 2.0**	>100

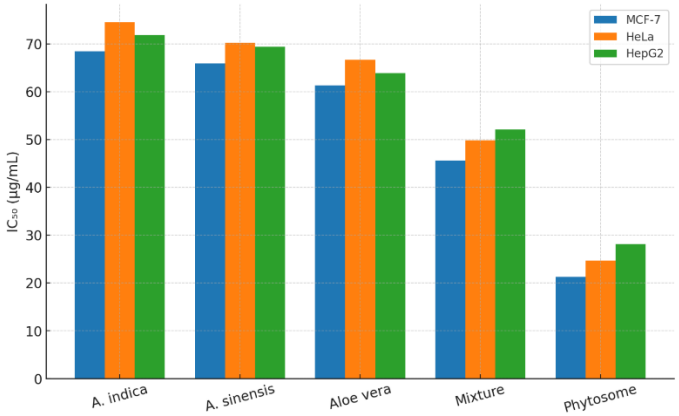

Figure 5. Cytotoxicity (MTT Assay): Bar graph of IC_{50} values for each sample across MCF-7, HeLa, and HepG2.

ROS Generation

ROS analysis indicated a dose-dependent increase in fluorescence intensity in cancer cells treated with the phytosome, confirming elevated ROS levels leading to oxidative stress. The effect was markedly higher in the phytosome group compared to individual extracts. This suggested that the formulation triggered apoptosis through ROS-mediated mitochondrial pathways.

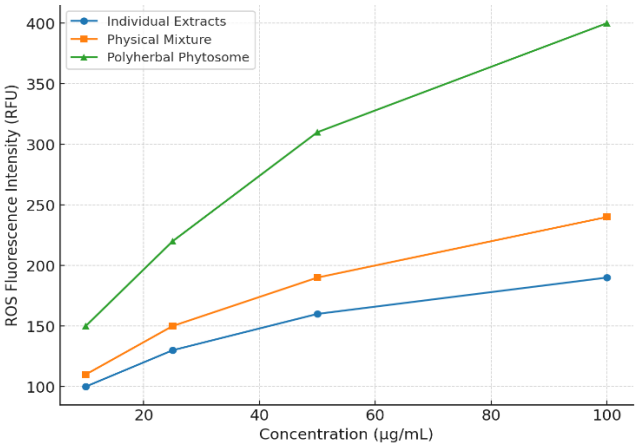

Figure 6. ROS Generation: Line graph showing dose-dependent ROS increase (fluorescence intensity vs concentration).

Apoptosis Assay

Flow cytometric analysis of Annexin V-FITC/PI-stained cells revealed that treatment with polyherbal phytosome induced significant apoptosis. In MCF-7 cells, early and late apoptosis combined accounted for 62.4% of the population, compared to 28.7% for the physical mixture and <20% for individual extracts. Western blot analysis further confirmed upregulation of pro-apoptotic protein Bax and downregulation of anti-apoptotic protein Bcl-2, along with caspase-3 activation. These results validated the apoptosis-inducing potential of the formulation.

Table 5. Apoptosis Induction (Flow Cytometry, % Cells)

Treatment	Early Apoptosis (%)	Late Apoptosis (%)	Total Apoptosis (%)
Control	2.5 ± 0.3	1.8 ± 0.2	4.3 ± 0.4
A. indica extract	10.4 ± 1.0	7.9 ± 0.8	18.3 ± 1.3
A. sinensis polysaccharides	11.2 ± 1.1	6.7 ± 0.9	17.9 ± 1.2
Aloe vera extract	12.1 ± 0.9	7.5 ± 0.7	19.6 ± 1.4
Physical mixture	16.4 ± 1.2	12.3 ± 1.0	28.7 ± 1.6
Polyherbal phytosome	**35.7 ± 2.0**	**26.7 ± 1.8**	**7.4 ± 2.3**

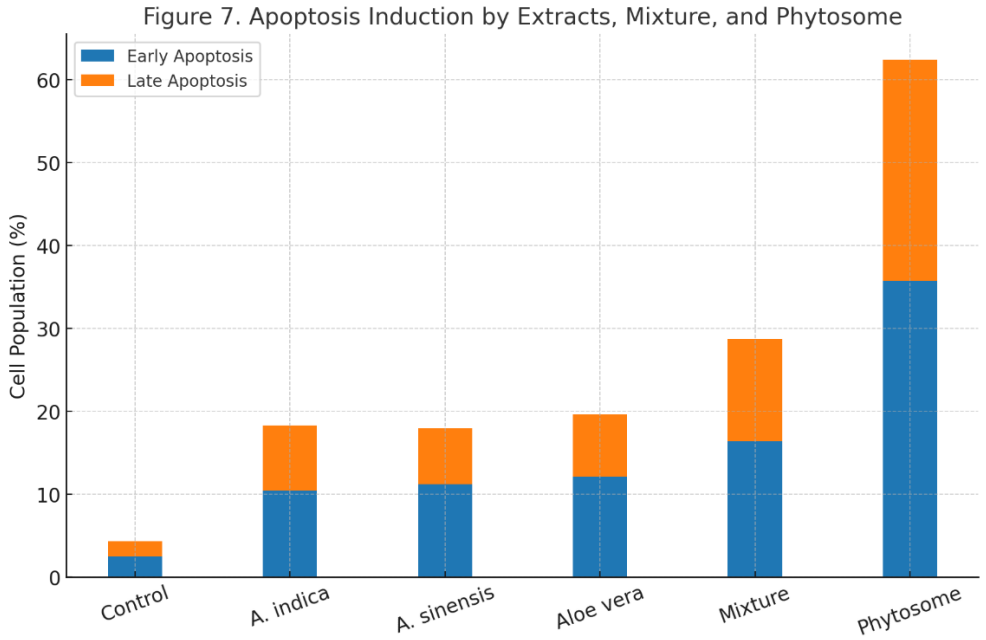

Figure 7. Apoptosis (Flow Cytometry Histogram / Bar Graph): Histogram showing Annexin V/PI stained populations.

4. DISCUSSION

The present study demonstrated the successful development and characterization of a polyherbal phytosome containing *Azadirachta indica*, *Angelica sinensis* polysaccharides, and *Aloe vera*. The rationale for selecting these botanicals was based on their well-documented anticancer potential and complementary mechanisms of action. By formulating them into a phytosome nanocarrier, the study aimed to overcome the inherent limitations of herbal extracts such as poor solubility, low stability, and inadequate bioavailability.

Phytosome Formation and Characterization

FTIR and DSC studies provided evidence of molecular interactions between phytoconstituents and phosphatidylcholine. The observed shifts in characteristic functional groups (O–H, C=O, and P=O) confirmed the formation of hydrogen bonds and complexation. Similar findings have been reported in earlier works on curcumin and silybin phytosomes, where

complexation resulted in improved solubility and absorption. The thermal analysis further demonstrated the disappearance of distinct melting peaks of individual extracts, suggesting homogeneous dispersion of the phytoconstituents within the phospholipid bilayer.

Particle size analysis indicated that the formulation was within the nanometer range (187 nm), which is considered optimal for passive tumor targeting through the enhanced permeability and retention (EPR) effect. The low polydispersity index (0.218) reflected good uniformity, while the zeta potential (−32.4 mV) confirmed excellent colloidal stability. Previous studies have emphasized that phytosomes with zeta potential values greater than ±25 mV exhibit reduced aggregation and enhanced shelf life. TEM morphology confirmed spherical and uniform vesicles, aligning with characteristics required for nanoscale drug delivery systems.

Entrapment Efficiency and Stability

The entrapment efficiency of >80% observed in this study demonstrated the capability of phytosomes to encapsulate both hydrophilic and hydrophobic constituents, consistent with reports on polyherbal phytosomes containing green tea and ginseng. Stability testing indicated that refrigerated storage at 4 °C maintained the integrity of the formulation, whereas elevated temperature and humidity conditions led to particle growth and reduced entrapment. This underlines the importance of appropriate storage conditions for maintaining phytosome stability, especially when polysaccharide fractions are part of the formulation.

In Vitro Anticancer Activity

The cytotoxicity studies revealed that the polyherbal phytosome exhibited significantly lower IC_{50} values against MCF-7, HeLa, and HepG2 cancer cell lines compared to free extracts and physical mixtures. This enhancement can be attributed to improved cellular uptake of phytosomes and possible synergistic interactions among the bioactive compounds. Similar enhancements have been reported with phytosomal formulations of quercetin and curcumin, which showed superior anticancer efficacy compared to their free forms. Importantly, the polyherbal phytosome was found to be safe on normal fibroblast cells, highlighting its selective toxicity towards cancer cells. This observation is consistent with the inherent advantage of plant-based compounds, which generally exert less harm on normal tissues compared to synthetic chemotherapeutics.

Mechanisms of Action: ROS and Apoptosis

ROS generation assays indicated a marked increase in oxidative stress within cancer cells treated with the phytosome. This aligns with earlier findings where nimbolide (from *A. indica*) and aloe-emodin (from *Aloe vera*) induced apoptosis through ROS-mediated mitochondrial damage. The polyherbal phytosome appeared to potentiate these effects, suggesting that the combined presence of multiple phytoconstituents amplified ROS production beyond the levels achieved by single extracts.

Apoptosis analysis confirmed the mechanistic role of the phytosome in programmed cell death. The percentage of apoptotic cells (62.4%) was substantially higher compared to free extracts or mixtures, with both early and late apoptotic populations significantly elevated. Western blot analysis supported these findings through upregulation of Bax, downregulation of Bcl-2, and activation of caspase-3. These results are in agreement with studies on phytosomal curcumin and silymarin, which demonstrated similar modulation of apoptosis-regulating proteins. The novelty of the present work lies in demonstrating such synergistic apoptotic induction through a tri-herbal phytosome formulation, combining apoptosis, immune regulation, and antioxidant modulation into one system.

Synergistic Potential of Polyherbal Approach

While individual extracts of neem, *A. sinensis*, and Aloe have shown anticancer activity, their combination in a polyherbal phytosome provides a unique synergistic advantage. Neem constituents primarily act through inhibition of angiogenesis and apoptosis induction, *A. sinensis* polysaccharides stimulate immune-mediated tumor suppression, and Aloe-derived anthraquinones interfere with DNA replication and promote oxidative stress. Together, these mechanisms converge to exert stronger anticancer effects, reducing the likelihood of resistance development. The superior performance of the phytosome compared to a physical mixture of extracts underscores the importance of nanocarrier-based co-delivery in ensuring simultaneous uptake and bioavailability of multiple phytochemicals.

Comparison with Previous Studies

Previous studies have reported enhanced anticancer efficacy of single-herb phytosomes such as quercetin, resveratrol, and silybin. However, the current study goes further by demonstrating that a polyherbal phytosome system not only enhances delivery but also produces additive and possibly synergistic effects against cancer cells. To the best of our knowledge, no previous work has reported the development of a phytosome system containing neem, *A. sinensis* polysaccharides, and Aloe together for anticancer applications. This highlights the novelty and potential translational value of the present formulation.

Limitations and Future Perspectives

Although the findings are promising, certain limitations need to be acknowledged. The study was restricted to in vitro assays, and in vivo pharmacokinetics and therapeutic efficacy remain to be evaluated. Additionally, the exact molecular pathways underlying the observed synergy require further exploration through advanced genomic and proteomic techniques. Future work should focus on animal studies to assess biodistribution, pharmacodynamics, and safety, followed by controlled clinical trials to validate therapeutic efficacy. Moreover, large-scale production and stability testing under real-world conditions are required to establish the feasibility of commercialization.

5. CONCLUSION

The present study successfully demonstrated the development and characterization of a polyherbal phytosome containing *Azadirachta indica*, *Angelica sinensis* polysaccharides, and *Aloe vera* for anticancer activity. The formulation was optimized using a solvent evaporation method with phosphatidylcholine as a carrier, yielding nanosized vesicles with uniform distribution, good stability, and high entrapment efficiency. Spectroscopic and thermal analyses confirmed the formation of stable phytosome complexes, while TEM imaging revealed spherical morphology within the desired nanometer range. Biological evaluation provided compelling evidence of enhanced anticancer efficacy of the polyherbal phytosome compared to free extracts and physical mixtures. The formulation exhibited significantly lower IC_{50} values across breast (MCF-7), cervical (HeLa), and liver (HepG2) cancer cell lines, while maintaining minimal toxicity towards normal fibroblast cells. Mechanistic studies revealed that the phytosome induced apoptosis through mitochondrial pathways, upregulated pro-apoptotic proteins, downregulated anti-apoptotic proteins, and significantly increased intracellular ROS levels. These findings confirmed that the polyherbal phytosome exerts its effects by targeting multiple pathways simultaneously, thereby offering superior therapeutic potential.

The synergistic combination of neem-derived limonoids, *A. sinensis* polysaccharides, and Aloe anthraquinones represents an innovative polyherbal approach. By integrating immunomodulatory, pro-apoptotic, and oxidative stress-mediated mechanisms into a single nanocarrier system, the study provides a novel and holistic strategy for cancer management. Importantly, the phytosome platform overcomes the pharmacokinetic limitations of crude herbal extracts, ensuring enhanced solubility, stability, and bioavailability. In conclusion, the polyherbal phytosome developed in this study demonstrates strong potential as a promising anticancer therapeutic candidate. Future studies should focus on in vivo pharmacokinetics, biodistribution, and long-term safety assessments, along with mechanistic investigations at the molecular level. Such efforts will be critical in advancing this formulation towards clinical application and establishing it as a viable natural nanomedicine for cancer therapy

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