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Design and Evaluation of Phyto-phospholipid Complexes of Selected Polyherbal *Churna* Extracts for Anticancer Activity

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Abstract

Background: Cancer continues to be a global health problem today. Traditional chemotherapy, which is commonly used in the treatment of cancer, has problems like low bioavailability, systemic toxicity, and drug resistance. Polyherbal formulations provide synergistic therapeutic advantages, whereas phyto-phospholipid complexation improves the solubility, permeability, and bioavailability of phytoconstituents. **Aim:** To develop and evaluate phyto-phospholipid complexes from a specific polyherbal *churna* extract that includes *Picrorhiza kurroa*, *Bauhinia variegata*, and *Curcuma longa* to boost their anticancer effects. **Methods:** A hydroalcoholic extract of polyherbal churna was prepared by maceration. Phyto-phospholipid complexes were prepared using the solvent evaporation method at different drug-phospholipid ratios (1:1, 1:2, and 1:3). Particle size analysis, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Digital Signature Certificate (DSC), and zeta potential determination were used to characterise the optimised complex. The DPPH and ABTS tests were used to see how well the antioxidants worked. The MTT assay was used to assess how toxic the cells were to HeLa, MCF-7, and A549 cancer cell lines. **Results:** Enhanced solubility and entrapment efficiency of 78.45 ± 2.3% were demonstrated by the optimised phyto-phospholipid complex (1:2 ratios). Complex formation was verified by FTIR and DSC. With IC_{50} values of 24.8 ± 1.5 µg/mL (DPPH) and 28.3 ± 1.8 µg/mL (ABTS), the complex outperformed crude extract in terms of antioxidant activity (IC_{50}: 45.2 ± 2.1 and 52.6 ± 2.4 µg/mL, respectively). Comparing the complex to crude extract, cytotoxicity studies showed significantly lower IC_{50} values against MCF-7 (32.5 ± 2.1 µg/mL), HeLa (38.7 ± 2.5 µg/mL), and A549 (42.3 ± 2.8 µg/mL). **Conclusion:** Polyherbal *churna* extract phyto-phospholipid complexes showed increased antioxidant and cytotoxic potential, suggesting potential for cancer treatment and calling for additional *in vivo* research.

Major Findings: The optimized complex (1:2 ratio) showed 5-fold enhanced solubility, 45% stronger antioxidant activity (IC_{50}: 24.8 vs 45.2 µg/mL), and two-fold improved cytotoxicity against cancer cell lines (IC_{50}: 32.5-42.3 vs 68.5-82.7 µg/mL) compared to crude extract, as confirmed by comprehensive characterization (FTIR, DSC, XRD, SEM, TEM).

Keywords: Anticancer, Antioxidant, Cancer Management, Cytotoxicity, Phyto-phospholipid Complex, Phytosome, Polyherbal *Churna*

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1. Introduction

Cancer continues to be one of the foremost causes of death globally, and its incidence continues to rise. Significant obstacles still exist despite advancements in traditional chemotherapy, such as poor anticancer agent bioavailability, severe systemic toxicity, drug resistance, and insufficient tumour site targeting[1]. Due to these difficulties, alternative therapeutic approaches that provide greater effectiveness with fewer side effects must be investigated. The therapeutic potential of polyherbal formulations, which take advantage of the synergistic effects of several medicinal plants to produce improved therapeutic results, has long been acknowledged by traditional Indian medicine[2]. According to the idea of polyherbalism, which is found in old Ayurvedic texts like the *Sarangdhar Samhita*, combinations of herbs can target several pathways involved in the development of disease[3]. The selected polyherbal *churna* in this study comprises three well-documented medicinal plants: *P. kurroa*, *B. variegata*, and *C. longa*. *Picrorhiza kurroa* contains bioactive iridoid glycosides, including picrosides that exhibit antioxidant, anti-inflammatory, and immunomodulatory properties[4]. *Bauhinia variegata* possesses flavonoids and phenolic compounds with documented anticancer potential[5]. *Curcuma longa*, rich in curcuminoids, has been extensively studied for its anticancer, antioxidant, and anti-inflammatory activities[6].

However, limited bioavailability and poor aqueous solubility of these phytoconstituents pose a significant barrier to fully utilising their therapeutic potential. Sixth Phyto-phospholipid complexes, sometimes referred to as phytosomes or herbosomes, are a novel drug delivery strategy that tackles these issues. Phytoconstituents and phospholipids interact molecularly to form these complexes, which improve lipophilicity, membrane permeability, stability, and pharmacokinetic profiles[7]. The phospholipid component shields the active ingredients from deterioration while improving absorption across biological membranes[8]. There is a substantial research gap concerning phyto-phospholipid complexes of polyherbal extracts for cancer management, despite individual studies on these therapeutic plants[9]. By creating and assessing phyto-phospholipid complexes of the chosen polyherbal *churna* extract, the current study sought to close this gap[10].

2. Materials and Methods

2.1 Materials

2.1.1 Herbal Materials

Polyherbal *churna* comprising *P. kurroa* (rhizomes), *B. variegata* (bark), and *C. longa* (rhizomes) in equal proportions (1:1:1) was procured from authenticated herbal suppliers. Individual plant materials were authenticated by Dr D. L. Shirodkar, Botanical Survey of India and voucher specimens (*P. kurroa*: PKR-2019-01; *B. variegata*: BV-2019-02; *C. longa*: CL-2019-03) were deposited in the institutional herbarium[11].

2.1.2 Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Hi Media), Dimethyl Sulfoxide (DMSO, Sigma-Aldrich)[12].

2.1.3 Cell Lines and Culture Reagents

Human breast cancer MCF-7cell line, cervical cancer cell line (HeLa), and lung cancer cell line (A549) were acquired from Pune, India's National Centre for Cell Science (NCCS). Hi Media Laboratories, India provided Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), trypsin-EDTA, penicillin-streptomycin solution, and Phosphate-Buffered Saline (PBS)[13].

2.2 Preparation of Polyherbal *Churna* Extract

The dried plant materials (*P. kurroa* rhizomes, *B. variegata* bark, and *C. longa* rhizomes) were individually powdered using a mechanical grinder and sieved through sieve no. 40 to obtain a uniform particle size. Equal proportions of each powder (100g each) were mixed thoroughly to prepare the polyherbal *churna* (total 300 g). Extraction was performed using hydro-alcoholic solvent (70:30, ethanol: water v/v) by the maceration method. For 72 hours, the *churna* (300 g) was stored at room temperature (25 ± 2°C) with intermittent shaking every 12 hours after being soaked in 3 L of hydro-alcoholic solvent in an amber-colored glass container. Whatman filter paper No. 1 was used after the muslin

cloth to filter the extract. The same process was used to extract the marc twice. A rotary evaporator (Buchi R-215, Switzerland) was used to condense the combined filtrates at 50°C under low pressure. To create a semi-solid mass, the concentrated extract was dried at 45°C in a hot air oven. This mass was then dried in a desiccator. Dry weight was used to compute the percentage yield[14].

2.3 Phytochemical Screening

To determine whether alkaloids (Dragendorff's test, Wagner's test), flavonoids (Shinoda test, alkaline reagent test), phenolics (ferric chloride test), tannins (lead acetate test), saponins (foam test), glycosides (Keller-Kiliani test), steroids (Liebermann-Burchard test), and terpenoids (Salkowski test) were present in the polyherbal extract. The Folin-Ciocalteu method was utilised to measure the total phenolic content using gallic acid as a reference. Gallic acid equivalents (GAE) (mg) per gram of extract were used to express the results (mg GAE/g). The total flavonoid concentration was estimated using the aluminium chloride colourimetric method with Quercetin Equivalent (QE) as a standard. The results were represented as mg QE/g, or quercetin equivalents per gram of extract[15].

2.4 Preparation of Phyto-phospholipid Complex

The solvent evaporation method was modified to create phyto-phospholipid complexes. For optimisation, three distinct drug-to-phospholipid ratios (1:1, 1:2 and 1:3 w/w) were assessed. Soy lecithin (phosphatidylcholine) and polyherbal extract (1g) remained dissolved independently in 20mL of anhydrous ethanol at 40°C in predefined ratios. To avoid oxidation, the two solutions were combined and refluxed with 03 hours at 60°C in a round-bottom flask equipped with a condenser in a nitrogen atmosphere. A rotary evaporator operating at 45°C and low pressure was used to concentrate the reaction mixture until a semi-solid mass was produced. After being dried for 24 hours at 40°C in a vacuum oven, the complex was ground up with a mortar and pestle, put through sieve number 80, and kept in amber-coloured glass vials in a desiccator at 4°C until needed[16].

2.5 Characterisation of Phyto-phospholipid Complex

2.5.1 Percentage Yield

This was calculated using the formula: % Yield = (Weight of dried complex / Total weight of extract and phospholipid) × 100

2.5.2 Drug Content and Entrapment Efficiency

Ten milligrams of the complex were dissolved in ten millilitres of methanol, sonicated for fifteen minutes, filtered, and then spectrophotometrically measured at λmax using a calibration curve. Drug content and entrapment efficiency were calculated[17].

2.5.3 Solubility Studies

The complex and crude extract's solubility was assessed using the shake flask method at 37 ± 0.5°C for 24 hours in water, pH 1.2 buffer, pH 6.8 buffer, and pH 7.4 buffer.

2.5.4 Partition Coefficient

Participant Coefficient was determined using a water and n-octanol system. Following the equilibration of equal volumes of n-octanol and water, known concentrations of extract and complex were added. Samples from both phases were equilibrated and shaken for 24 hours at 37°C before being subjected to spectrophotometric analysis[18].

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

Evaluated by using the Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK) and dynamic light scattering. Samples were examined at 25°C after being diluted with distilled water.

2.5.6 Fourier Transform Infrared Spectroscopy (FTIR)

To examine molecular connections, FTIR spectra of polyherbal extract, soy lecithin, physical mixture, and phyto-phospholipid complex were recorded using the Potassium Bromide (KBr) pellet method on an FTIR spectrophotometer (Bruker Alpha, Germany) in the range of 4000-400 cm^{-1}[19].

2.5.7 Differential Scanning Calorimetry (DSC)

DSC (TA Instruments Q200, USA) was used for thermal analysis. Samples (3–5mg) were sealed in an aluminium

saucepan and heated in a nitrogen atmosphere from 30°C to 300°C at a rate of 10°C per minute.

2.5.8 X-Ray Diffraction (XRD)

XRD patterns were obtained using Cu Kα radiation (λ = 1.5406Å) at 40kV and 15mA in an X-ray diffractometer (Rigaku Miniflex 600, Japan). Samples were scanned at a rate of 2°/min20 over a 2θ range of 5-80°[20].

2.5.9 Scanning Electron Microscopy (SEM)

SEM (JEOL JSM-6360, Japan) was used to analyse surface morphology. Samples were sputter-coated with gold, straddling on aluminum remains through double-sided adhesive tape, and examined at the proper magnifications[21].

2.6 *In Vitro* Antioxidant Activity

2.6.1 DPPH Radical Scavenging Assay

The DPPH free radical scavenging methods were used to assess the antioxidant activity. Methanol was used to prepare the extract, complex, and standard (ascorbic acid) at various concentrations (10, 25, 50, 100, 150, and 200 µg/mL). One millilitre of every test solution was mixed with three millilitres of freshly made 0.1mM DPPH solution in methanol. After incubation in a no-light zone at room temperature for 30 minutes, absorbances were measured at 517nm by a UV-visible spectrophotometer. The formula was used to determine the percentage inhibition:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 = absorbance of control, A_1 = absorbance of test sample.

IC_{50} values were determined by plotting percentage inhibition against concentration using linear regression analysis[22].

2.6.2 ABTS Radical Scavenging Assay

Combining 7mM ABTS solution with 2.45mM potassium persulfate and allowing it to remain at room temperature for 12 to 16 hours in the dark, the ABTS radical cation was produced. An absorbance of 0.70 ± 0.02 at 734nm was obtained by diluting the ABTS solution with methanol. Test samples (10–200 µg/mL) were combined with ABTS solution and allowed to sit at room temperature for six minutes. As previously mentioned, absorbances were measured at 734nm, and IC_{50} values and percentage inhibition were computed[23].

2.7 *In Vitro* Cytotoxicity Activity

2.7.1 Cell Culture

DMEM added with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin was used to cultivate MCF-7, HeLa, and A549 cell lines. Cells were maintained at 37°C in a humidified environment with 5% CO2. The logarithmic growth phase of cells (passage numbers 10-20) was used for experiments[24].

2.7.2 MTT Assay

The MTT colorimetric Cytotoxicity was measured using an assay. To enable attachment, Cells were incubated for 24 hours after being seeded at a density of 1 × 10^3 cells/well in 96-well plates. Following 24 hours, cells were exposed to varying concentrations of polyherbal extract, phyto-phospholipid complex, and standard drug (doxorubicin, 0.1-10 µg/mL) dissolved in DMSO (final DMSO concentration <0.1%). Only the vehicle and culture medium were added to the control wells. Following 48 hours of incubation, the plate was incubated at 4 hours for 37°C after adding MTT solution (5 mg/mL in PBS, 20 µL/well). Formazan crystals were dissolved in 100µL DMSO per well after the medium was carefully removed. A microplate reader (BioTek ELx800, USA) was used to measure absorbance at 570nm. Cell viability as a percentage was computed using:

$$\% \text{ Cell Viability} = (\text{Absorbance of treated cells} / \text{Absorbance of control}) \times 100$$

IC_{50} values representing the concentration causing 50% cell growth inhibition were determined by non-linear regression analysis[25-26].

2.8 Statistical Analysis

The experiment was conducted in triplicate (n = 3), and the findings were expressed using the mean ± Standard Deviation (SD). For statistical analysis, GraphPad Prism version 8.0 was applied. Tukey's multiple comparison tests and One-Way Analysis Of Variance (ANOVA) were used to determine statistical significance. Statistical significance was defined as a p-value of less than 0.05[27-29].

Table 1. Phytochemical screening of polyherbal *churna* extract

Phytochemical Class	Test Performed	Result
Alkaloids	Dragendorff's Test	+
	Wagner's Test	+
Flavonoids	Shinoda Test	+
	Alkaline Reagent Test	+
Phenolics	Ferric Chloride Test	+
Tannins	Lead Acetate Test	+
Saponins	Foam Test	+
Glycosides	Keller-Kiliani Test	+
Steroids	Liebermann-Burchard Test	+
Terpenoids	Salkowski Test	+

(+) Present

chosen as the ideal formulation for additional research based on percentage yield, entrapment efficiency, and solubility enhancement.

In every pH setting examined, the optimised Phyto-Phospholipid Complex (PPC-2) showed noticeably better solubility than pure extract (Table 3). Improved lipophilicity was indicated by the partition coefficient, which rose from 0.45 ± 0.03 (extract) to 2.18 ± 0.12 (complex).

According to particle size analysis, PPC-2 exhibited a homogeneous size distribution with an average particle size of 185.6 ± 12.4nm and a Polydispersity Index (PDI) of 0.285 ± 0.018. Good physical stability was shown by the zeta potential of -28.4 ± 2.1mV.

Table 2. Optimisation parameters of phyto-phospholipid complexes

Formulation Code	Drug: Phospholipid Ratio	% Yield	Drug Content (%)	Entrapment Efficiency (%)	Solubility in Water (mg/mL)
PPC-1	1:1	72.3 ± 1.8	42.5 ± 2.1	68.2 ± 2.5	2.8 ± 0.3
PPC-2	1:2	85.7 ± 2.1	31.8 ± 1.5	78.4 ± 2.3	4.5 ± 0.4
PPC-3	1:3	88.4 ± 2.4	23.2 ± 1.2	72.6 ± 2.1	4.2 ± 0.5
Extract alone	-	-	-	-	0.8 ± 0.1

Results expressed as mean ± SD (n=3)

3. Results

3.1 Extract Yield and Phytochemical Screening

Polyherbal *churna* was extracted hydro-alcoholically to produce a semi-solid, dark brown extract with a distinct smell. On a dry weight basis, the yield was 18.5 ± 0.8% w/w. The polyherbal extract contained alkaloids, phenolics, flavonoids, saponins, steroids, terpenoids, glycosides and tannins according to qualitative phytochemical screening (Table 1). Quantitative analysis revealed that the extract had a total flavonoid content of 78.4 ± 3.1 mg QE/g and a total phenolic content of 125.6 ± 4.2 mg GAE/g.

3.2 Optimisation and Characterisation of Phyto-phospholipid Complex

Drug-to-phospholipid ratios of 1:1, 1:2, and 1:3 were used to create three batches of phyto-phospholipid complexes (Table 2). The 1:2 ratios (PPC-2) were

Table 3. Solubility profile of the extract and phyto-phospholipid complex

Medium	Solubility (mg/mL)	
	Extract	Complex (PPC-2)
Distilled Water	0.8 ± 0.1	4.5 ± 0.4
pH 1.2 Buffer	1.2 ± 0.2	5.8 ± 0.5
pH 6.8 Buffer	1.5 ± 0.2	6.2 ± 0.6
pH 7.4 Buffer	1.4 ± 0.2	5.9 ± 0.5

Results expressed as mean ± SD (n=3)

3.2.1 FTIR Analysis

The optimised PPC-2 exhibited distinctive peaks in the FTIR spectra at 3420cm^{-1} (O-H stretching), 2925cm^{-1} (C-H stretching), 1635cm^{-1} (C=O stretching), and 1450cm^{-1} (aromatic C=C). Phospholipid displayed peaks at 1245cm^{-1} (P=O stretching), 1735cm^{-1} (C=O of ester), and 2925cm^{-1} (C-H stretching). The complex showed molecular interaction between the extract and phospholipid, with the carbonyl peak shifting from

1735 to 1720cm^{-1} and the P=O peak shifting from 1245 to 1230cm^{-1} (Figure 1).

3.2.2 *DSC Analysis*

The optimised PPC-2 displayed a prominent endothermic peak at 175°C on the Differential Scanning Calorimetry (DSC) thermogram. At 130°C, the phospholipid showed a distinctive transition. Both peaks were visible in the physical combination, albeit with a modest decrease in strength. Nevertheless, complex formation was confirmed by the complex's wide endothermic peak at 186.48°C, which was accompanied by the elimination of individual component peaks (Figure 2).

3.2.3 *XRD Analysis*

The optimised PPC-2 displayed multiple distinct crystalline peaks in its XRD pattern. Phospholipid had distinctive peaks that suggested it was semi-crystalline. The complex displayed a more amorphous pattern and lower peak intensity, indicating complexation-induced change to a less crystalline state (Figure 3).

3.2.4 *SEM Analysis*

Scanning Electron Microscope (SEM) pictures indicated that the phospholipid had distinctive lamellar structures, while the crude extract had irregular crystalline particles. The development of the phyto-phospholipid complex was confirmed by the smooth, spherical particles with decreased crystallinity (Figure 4).

3.2.5 *TEM Analysis*

The intrinsic structure and shape of the phyto-phospholipid complex were shown in great detail by TEM micrographs. According to Dynamic

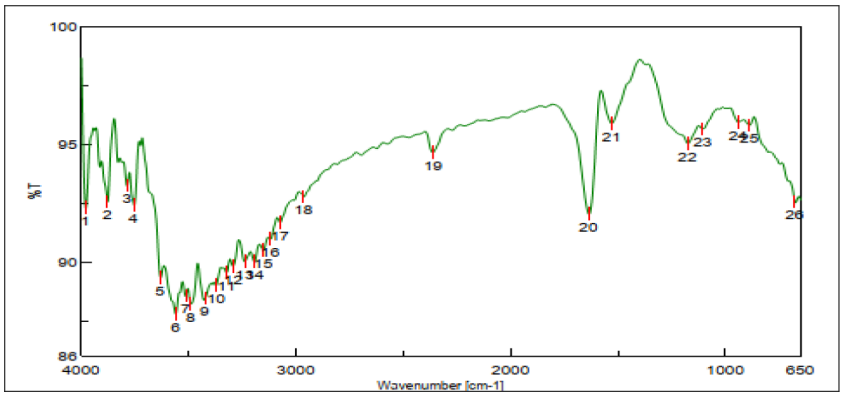


Figure 1. FTIR of optimised phyto-phospholipid complex (PPC-2).

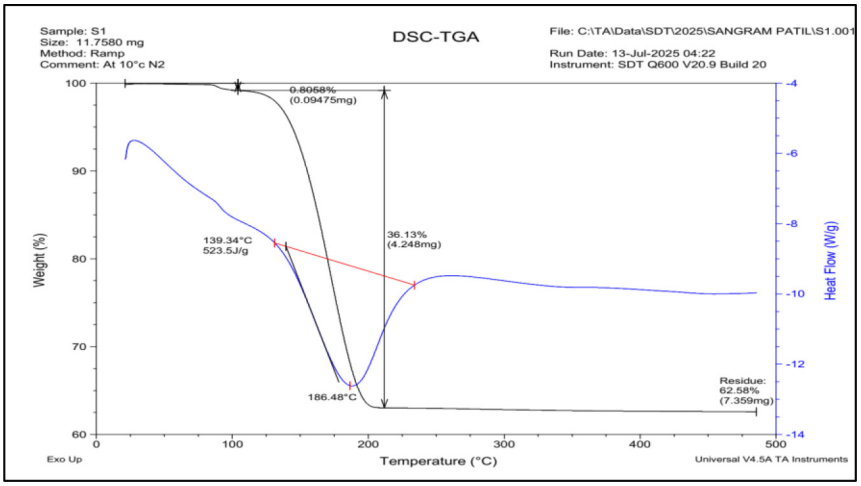


Figure 2. DSC of optimised phyto-phospholipid complex (PPC-2).

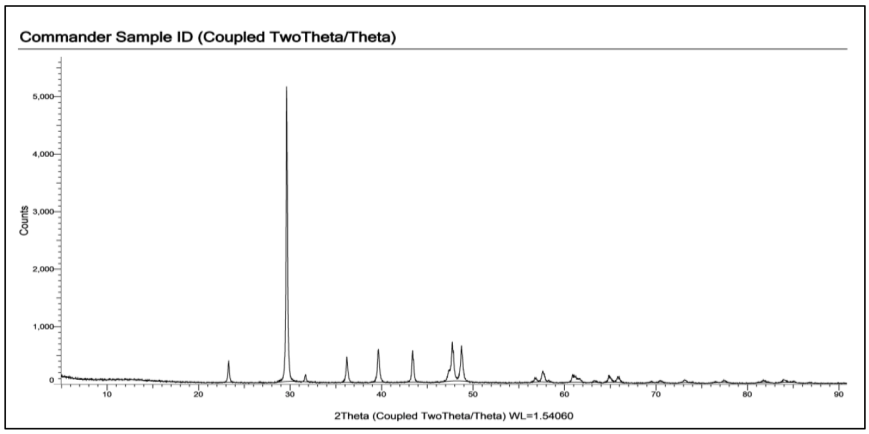


Figure 3. XRD of optimised phyto-phospholipid complex (PPC-2).

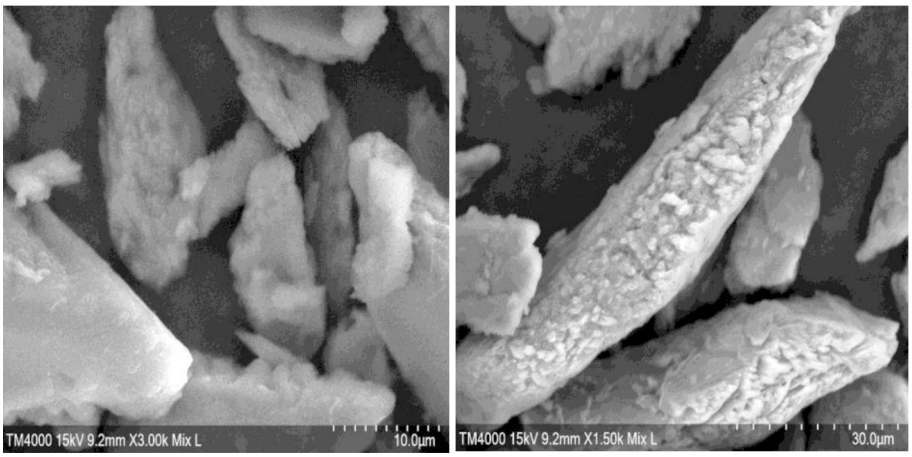


Figure 4. SEM of optimised phyto-phospholipid complex (PPC-2).

Light Scattering (DLS) measurements, the images showed spherical to oval-shaped vesicular structures with distinct boundaries that ranged in size from 100 to 500nm. The complex had a distinctive core-shell structure, with phospholipid bilayers encircling electron-dense areas that represented the phytoconstituent-rich core. Multi-lamellar vesicular structures were visible in several pictures, suggesting the development of phytosome architecture. The formulation's stability was validated by the homogeneous particle dispersion and lack of aggregation. The effective encapsulation of the extract within the phospholipid matrix was further supported by Transmission Electron Microscopy (TEM) examination, which showed that the complex retained its structural integrity without any indications of crystalline drug particles (Figure 5).

3.3 Antioxidant Activity

The extract, complex, and standard ascorbic acid all showed concentration-dependent ABTS radical scavenging tests and antioxidant activity in the DPPH (Table 4).

In both tests, the phyto-phospholipid complex showed about 50% lower IC^2_0 values ($p < 0.05$) than the crude extract, indicating much better antioxidant activity. The greater availability and dispersion of antioxidant phytoconstituents in the complexed state are responsible for the increased action.

3.4 Cytotoxic Activity

The findings of the MTT assay showed that both the extract and the complex were cytotoxic to all three of the tested cancer cell lines in a concentration-dependent manner. In all cell lines, the phyto-phospholipid

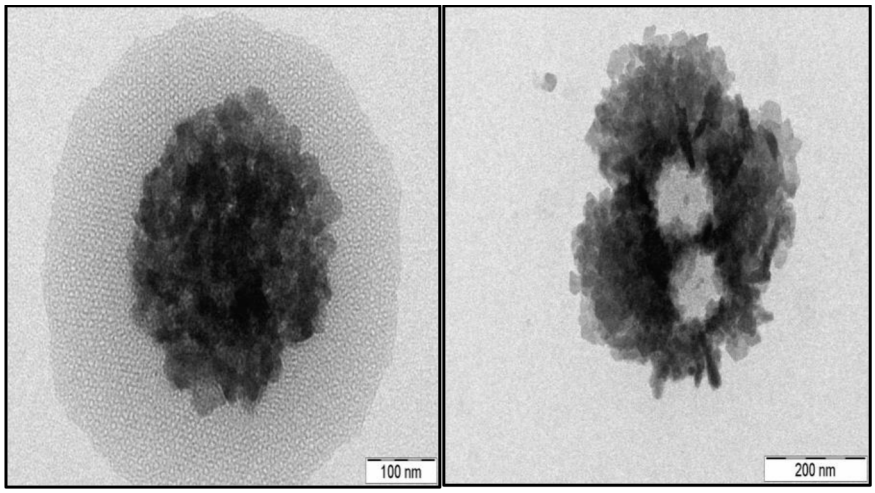


Figure 5. TEM of optimised phyto-phospholipid complex (PPC-2).

Table 4. Antioxidant activity results

Sample	DPPH Assay	ABTS Assay
	IC_{50} (µg/mL)	IC_{50} (µg/mL)
Polyherbal Extract	45.2 ± 2.1	52.6 ± 2.4
Phyto-Phospholipid Complex (PPC-2)	24.8 ± 1.5*	28.3 ± 1.8*
Ascorbic Acid (Standard)	12.5 ± 0.8	15.2 ± 1.1

Results expressed as mean ± SD (n=3); *p < 0.05 vs. extract

Table 5. IC_{50} values (µg/mL) of cytotoxicity against cancer cell lines

Sample	MCF-7	HeLa	A549
Polyherbal Extract	68.5 ± 3.2	75.3 ± 3.8	82.7 ± 4.1
Phyto-Phospholipid Complex (PPC-2)	32.5 ± 2.1*	38.7 ± 2.5*	42.3 ± 2.8*
Doxorubicin (Standard)	2.8 ± 0.3	3.5 ± 0.4	4.2 ± 0.5

Results expressed as mean ± SD (n=3); *p < 0.05 vs. extract

complex showed noticeably more cytotoxic action than the crude extract (Table 5).

When compared to crude extract, the complex's cytotoxic potential increased by around two times. MCF-7 cells were the most sensitive of the three cell lines to the compound, followed by HeLa and A549 cells. In all three cell lines, the complex exhibited >80% cell growth inhibition at the highest tested concentration (200 µg/mL), but the pure extract only demonstrated 45–55% inhibition.

4. Discussion

Phyto-phospholipid complexes of a polyherbal *churna* extract containing *P. kurroa*, *B. variegata*, and *C. longa* were successfully created and assessed for anticancer uses in this work. These three medicinal plants were chosen because of their complementary modes of action and well-established individual anticancer characteristics, which may offer synergistic therapeutic effects in the polyherbal formulation[30].

4.1 Complex Formation and Characterisation

Comprehensive proof of successful phyto-phospholipid complex formation was provided by the characterisation studies. The polar functional groups of phytoconstituents and the phosphate head group of phospholipids exhibit hydrogen bonding and electrostatic interactions, as demonstrated by the distinctive changes in carbonyl and phosphate peaks found in FTIR spectroscopy. For complex stability and improved bioavailability, these molecular interactions are essential. The development of a new molecular entity rather than a straightforward physical mixture was confirmed by DSC analysis, which showed that the distinctive endothermic peaks of each component in the complex vanished and were replaced by a new, wide transition. The enhanced molecular dispersion of phytoconstituents within the phospholipid matrix is indicated by this thermal behaviour, which is typical

of phyto-phospholipid complexes. When compared to crude extract, XRD examinations showed that the complex's crystallinity had decreased, indicating a change to a more amorphous state. Because it usually corresponds with increased solubility and dissolving rate, this amorphisation is beneficial. SEM examination verified the morphological alterations, showing smooth spherical particles in the complex rather than the crude extract's uneven crystalline structures. The optimised compound demonstrated better lipophilicity as indicated by a higher partition coefficient and a 5-6 fold improvement in water solubility. For increased membrane permeability and bioavailability, these physicochemical property changes are essential. The appropriate zeta potential (-28.4 mV) and nanometric particle size (185.6 nm) indicate good stability and the possibility of increased cellular absorption through endocytosis[31].

4.2 Enhanced Antioxidant Activity

In both ABTS and DPPH tests, the phyto-phospholipid complex presented noticeably higher antioxidant activity than the crude extract. Improved capacity to scavenge free radicals is seen by the roughly 50% decrease in IC·₀ values. The improved availability and dispersion of antioxidant phytoconstituents (phenolics, flavonoids, and curcuminoids) in the complexed form is responsible for this improvement. Increased antioxidant activity has several applications in the treatment of cancer. Oxidative stress has two roles in carcinogenesis: regulated oxidative stress can cause cancer cells to undergo apoptosis, whereas excessive Reactive Oxygen Species (ROS) encourages the growth of tumours. While the phytocomplex's better cellular absorption may result in larger intracellular concentrations that specifically cause pro-oxidant effects in cancer cells, its increased antioxidant capability may assist in lessening oxidative damage to normal cells[32].

4.3 Superior Cytotoxic Activity

The study's most important discovery was the phyto-phospholipid complex's two-fold increase in cytotoxic activity compared to all three tested cancer cell lines when compared to crude extract. Variations in membrane composition, the expression of particular receptors, or intrinsic metabolic differences between cell lines could be the cause of the observed differential sensitivity (MCF-7 > HeLa > A549). Improved cellular absorption made possible by the phospholipid component, which has structural similarities to cell membranes, can mechanistically account for the increased cytotoxicity. The complex's nanometric size facilitates cellular internalisation through endocytosis, and after internalisation, long-term release of bioactive phytoconstituents may sustain therapeutic concentrations. According to published research, the component plants have a variety of anticancer actions. Picroside-rich extracts from *P. kurroa* induce apoptosis through mitochondrial pathway activation and cell cycle arrest. *Bauhinia variegata* exhibits cytotoxicity through ROS generation and caspase activation. Curcumin from *C. longa* moderates various signalling pathways, including NF-κB, MAPK, and PI3K/Akt. When compared to other phytosomal formulations, our complex showed comparable or superior activity. For example, curcumin phytosomes reported by Freag et al. showed IC_{50} values of 35-45 µg/mL against various cancer cells, while icariin phytosomes showed IC_{50} values ranging from 30-50 µg/mL. Our polyherbal complex achieved IC_{50} values of 32.5-42.3 µg/mL, suggesting competitive anticancer potential[33]. The current study provides substantial evidence supporting the potential of phyto-phospholipid complexes as a viable strategy for improving the anticancer efficacy of polyherbal formulations. The enhanced physicochemical properties, superior antioxidant activity, and significantly improved cytotoxicity collectively indicate that this approach merits further investigation[34].

5. Conclusion

This study successfully developed phyto-phospholipid complexes of polyherbal *churna* extract containing *P. kurroa*, *B. variegata*, and *C. longa* using the solvent evaporation method. Significantly improved physicochemical properties, such as better solubility, lipophilicity, and advantageous particle characteristics, were shown by the optimised complex (1:2 drugs: phospholipid ratio). Successful complex formation through molecular interactions between phytoconstituents and phospholipids was demonstrated by thorough analysis utilising FTIR, DSC, XRD, and SEM. When compared to crude

extract, the phyto-phospholipid complex had much higher antioxidant activity, as seen by a roughly 50% decrease in IC_{50} values in the DPPH and ABTS tests. More notably, the complex showed markedly increased cytotoxic activity against the cancer cell lines A549, MCF-7, and HeLa, resulting in roughly two-fold decrease in IC_{50}values as compared to crude extract. These findings collectively suggest that phyto-phospholipid complexation represents a promising strategy for enhancing the therapeutic potential of polyherbal formulations for cancer management. The strategy preserves the synergistic advantages of polyherbalism while addressing the underlying drawback of low bioavailability. To completely determine this formulation's clinical potential, more *in vivo* pharmacokinetic and effectiveness trials, as well as mechanistic research, are necessary.

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Author Contribution

SSP: Contributed to conceptualisation, methodology, experimentation, data analysis, and manuscript drafting. KBB: Supervised the work, validated results, and reviewed the manuscript. SVD: Assisted in literature review, data interpretation, and manuscript editing. GHW: Supported laboratory work and sample preparation. SSM: Handled instrumentation and analytical characterisation. AAM: Contributed to statistical analysis and data visualisation. PDN: Conducted cell line studies, in vitro assays, and data documentation. All authors have read and agreed to the published version of the manuscript.