

FORMULATION AND CHARACTERIZATION OF PHOSPHOLIPID COMPLEX OF MEDICINAL PLANT EXTRACT *PIPER CUBEBA*

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ABSTRACT

This study investigates the formulation and characterization of a phospholipid complex of *Piper cubeba* extract to enhance its bioavailability, stability, and controlled release. The hydroalcoholic extract of *Piper cubeba* was prepared, and its phytochemical profile revealed the presence of flavonoids, phenols, and tannins, which contribute to its therapeutic properties. The extract was then encapsulated in phospholipid complexes, with formulation F10 being optimized for particle size (260.35 nm) and entrapment efficiency (76.65%). The in-vitro drug release studies indicated a sustained release over 12 hours, with 98.85% of the extract being released. The release pattern followed the Korsmeyer-Peppas model ($R^2 = 0.9719$), suggesting a combination of diffusion and erosion-controlled release mechanisms. This phospholipid complex formulation offers a promising approach to improve the pharmacokinetic properties of *Piper cubeba* and its potential use in therapeutic applications.

Keywords: *Piper cubeba*, phospholipid complex, bioactive compounds, drug release, entrapment efficiency, sustained release, phytochemical screening, formulation optimization, liposomal delivery.

INTRODUCTION

Phospholipids are crucial components of biological membranes, known for their amphiphilic properties, which allow them to interact with both hydrophobic and hydrophilic substances. Due to their versatile structure, phospholipids have been extensively utilized in the formulation of lipid-based drug delivery systems, such as liposomes, nanostructured lipid carriers (NLCs), and solid lipid nanoparticles (SLNs). These systems are particularly valuable in enhancing the bioavailability and stability of poorly water-soluble compounds, such as those derived from medicinal plants. Phospholipid complexes, in which active ingredients from plant extracts are

encapsulated within a phospholipid matrix, offer promising advantages in overcoming the challenges related to drug delivery, including poor solubility, instability, and low bioavailability.

Piper cubeba, commonly known as cubeb pepper, is a tropical plant belonging to the Piperaceae family. Traditionally used in various regions of Asia, *Piper cubeba* is renowned for its medicinal properties, including its anti-inflammatory, antimicrobial, antioxidant, and antidiabetic effects ¹. The primary bioactive compounds in *Piper cubeba* include essential oils, flavonoids, alkaloids, and terpenoids, which contribute to its therapeutic potential. However, these bioactive compounds face limitations such as poor solubility and stability when administered in their raw plant extract form, which may hinder their therapeutic efficacy.

In recent years, the formulation of phospholipid complexes containing medicinal plant extracts has emerged as a promising strategy to enhance the pharmacological potential of such compounds. By encapsulating *Piper cubeba* extract within phospholipids, it is possible to improve its solubility, stability, and controlled release properties. Phospholipid complexes can protect the active constituents from environmental factors such as light and oxygen, reduce their degradation, and provide a more effective release profile ². Furthermore, phospholipid-based delivery systems can facilitate the enhanced absorption of these compounds, particularly when targeting specific tissues or overcoming physiological barriers, such as the gastrointestinal tract or the blood-brain barrier ³.

The preparation of phospholipid complexes typically involves various techniques such as thin-film hydration, solvent evaporation, or high-pressure homogenization. These methods allow for the formation of nanoparticles, liposomes, or other self-assembled structures, which can encapsulate the bioactive compounds effectively. The characterization of these systems is vital for understanding their physicochemical properties and ensuring their stability, safety, and efficacy. Characterization methods commonly used for phospholipid complexes include particle size analysis, zeta potential measurement, and electron microscopy ⁴. These techniques help assess the encapsulation efficiency, release kinetics, stability, and the interactions between the plant extract and the phospholipid matrix.

Given the growing interest in plant-based therapeutics, the formulation of phospholipid complexes with *Piper cubeba* extract is a promising area of research. The development of such delivery systems could significantly enhance the therapeutic efficacy of *Piper cubeba*, making it a more viable option for treating a variety of diseases. This research aims to explore the formulation

strategies, physicochemical characterization, and potential benefits of phospholipid complexes of *Piper cubeba* extract for improved drug delivery.

Material and Methods:

Material

The chemicals used in our research are sourced from reputable suppliers, including Loba Chemie Pvt. Ltd., Thomas Baker, S. D. Fine Chem. Ltd., and Qualigens Fine Chemicals. These include essential reagents such as Potassium Mercuric Iodide, Iodine, Picric Acid, Ferric Chloride, and Methanol, which are used for a variety of chemical analyses and synthesis processes. The chemicals are crucial for conducting experiments like qualitative analysis, extractions, and the preparation of solutions in the laboratory.

Methods

Extraction by maceration process

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs ⁵. 70 gram plant materials of *Piper cubeba* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration. The extraction was continued till the defatting of the material had taken place. Defatted dried plant material of *Piper cubeba* were extracted with hydroalcoholic solvent (ethanol: water: 75:25 v/v) using maceration. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract.

Determination of Percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Qualitative phytochemical tests

Phytochemical examinations were carried out extracts as per the following standard methods ⁶.

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method ⁷. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared

in methanol. 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of Phenol. 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method ⁸. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Formulation development of phospholipids complex

The complex was prepared with phospholipids: cholesterol and *Piper cubeba* in the ratio of 1:1:1, 1:2:1, 2:1:1, 2:3:1 respectively. Weight amount of extract and phospholipids and cholesterol were placed in a 100ml round-bottom flask and 25ml of dichloromethane was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring.

The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle ⁹.

Table 1: Different formulations of phospholipids complex

Formulation	Ratio of Phospholipids and Cholesterol	Extract Concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:1	1	20
F2	1:2	1	20
F3	2:1	1	20
F4	2:3	1	20
Optimization of Drug Concentration			
F5	2:1	0.5	20
F6	2:1	1.0	20
F7	2:1	1.5	20
F8	2:1	2.0	20
Optimization of solvent concentration			
F9	2:1	1.0	5
F10	2:1	1.0	10
F11	2:1	1.0	15
F12	2:1	1.0	20

Characterization of prepared phospholipids complex

Microscopic observation of prepared phospholipids complex

An optical microscope (Cippon, Japan) with a camera attachment was used to observe the shape of the optimized phospholipids complex formulation ¹⁰.

Entrapment efficiency

Phospholipids complex preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4°C ¹¹. The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non-entrapped *Piper cubeba* was recorded at λ_{max} 420.0 nm using UV/visible spectrophotometer

(Labindia 3000+). Sediment was treated with 1ml of 0.1 % Triton x 100 to lyse the vesicles and diluted to 100 ml with 0.1 N HCl and absorbance taken at 420.0 nm. Amount of quercetin in supernatant and sediment gave a total amount of *Piper cubeba* in 1 ml dispersion. The percent entrapment was calculated by following formula:

$$\text{Percent Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \times 100$$

Particle size and size distribution

The particle size, size distribution of optimized phospholipids complex formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK). The electric potential of the phospholipids complex, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell ¹².

Transmission electron microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungstic acid ¹³. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy.

***In vitro* dissolution rate studies**

In vitro drug release of the sample was carried out using USP- type II dissolution apparatus (Paddle type) ¹³. The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5°C and 75 rpm. 10 mg of prepared phospholipids complex was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37°C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy.

RESULTS AND DISCUSSION

The results presented above provide insights into the formulation, characterization, and drug release behavior of the phospholipid complex of *Piper cubeba* extract.

The hydroalcoholic extract yielded 8.5%, which is a standard yield for plant-based extractions using hydroalcoholic solvents. This suggests an efficient extraction process that allows for the isolation of bioactive compounds from *Piper cubeba*.

The phytochemical screening revealed the presence of flavonoids, phenols, and tannins in the hydroalcoholic extract, as indicated by positive tests using lead acetate, ferric chloride, and gelatin, respectively. However, alkaloids, glycosides, proteins, carbohydrates, saponins, and diterpenes were absent in this extract. The presence of flavonoids, phenols, and tannins is noteworthy, as these compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which contribute to the medicinal potential of *Piper cubeba*. The absence of other bioactive compounds may guide further research into optimizing extraction methods to maximize the diversity of phytochemicals.

The quantification of total phenol and flavonoid content in the hydroalcoholic extract showed values of 0.632 mg/100mg and 0.874 mg/100mg, respectively. These values highlight the significant bioactive potential of the extract, with phenols and flavonoids being responsible for much of the plant's therapeutic properties. Phenolic compounds have been extensively studied for their role in protecting against oxidative stress and chronic diseases.

The particle size and entrapment efficiency of various phospholipid complexes were evaluated for their ability to encapsulate the hydroalcoholic extract of *Piper cubeba*. The optimized formulation (F10) demonstrated a particle size of 260.35 nm and an entrapment efficiency of 76.65%, which is promising. Smaller particle sizes generally improve the solubility and bioavailability of the active ingredients by enhancing surface area and permeability. The high entrapment efficiency indicates that a substantial amount of the bioactive compounds from the *Piper cubeba* extract were successfully incorporated into the phospholipid matrix, ensuring the stability and controlled release of the active ingredients.

The drug release profile for formulation F10 was determined, and the cumulative drug release over time demonstrated a sustained release pattern, with 98.85% of the extract released after 12 hours. This suggests that the phospholipid complex can effectively control the release of bioactive compounds, which could be advantageous for achieving prolonged therapeutic effects. The regression analysis of release data indicates that the release of the extract from formulation F10 follows a Korsmeyer-Peppas model ($R^2 = 0.9719$), implying that the drug release is controlled by

both diffusion and erosion mechanisms, characteristic of complex formulations such as liposomes or lipid-based systems.

Table 2: Result of percentage yield of extract

S. No.	Extract	Percentage yield (%)
1.	Hydroalcoholic	8.5%

Table 3: Result of phytochemical screening of *Piper cubeba* extract

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	Wagner's test	-ve
2.	Glycosides	
	Legal's test	-ve
3.	Flavonoids	
	Lead acetate	+ve
	Alkaline test	+ve
4.	Phenol	
	Ferric chloride test	+ve
5.	Proteins	
	Xanthoproteic test	-ve
6.	Carbohydrates	
	Fehling's test	-ve
7.	Saponins	
	Foam test	-ve
8.	Diterpenes	
	Copper acetate test	-ve
9.	Tannins	
	Gelatin Test	+ve

Abbreviation: +ve indicate presence, -ve indicate absence of phytochemicals

Table 4: Total bioactive constituents content of *Piper cubeba*

S. No.	Extract	Total phenol (mg/100mg)	Total Flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.632	0.874

Table 5: Particle size and entrapment efficiency of phospholipids complex

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	315.56	59.85
F2	356.65	64.58
F3	285.65	74.45
F4	315.65	65.45
F5	325.45	66.65
F6	285.65	74.45
F7	336.65	68.78
F8	336.68	66.74
F9	330.25	68.85
F10	260.35	76.65
F11	315.25	66.45
F12	285.65	74.45

Average of three determinations (n=3)

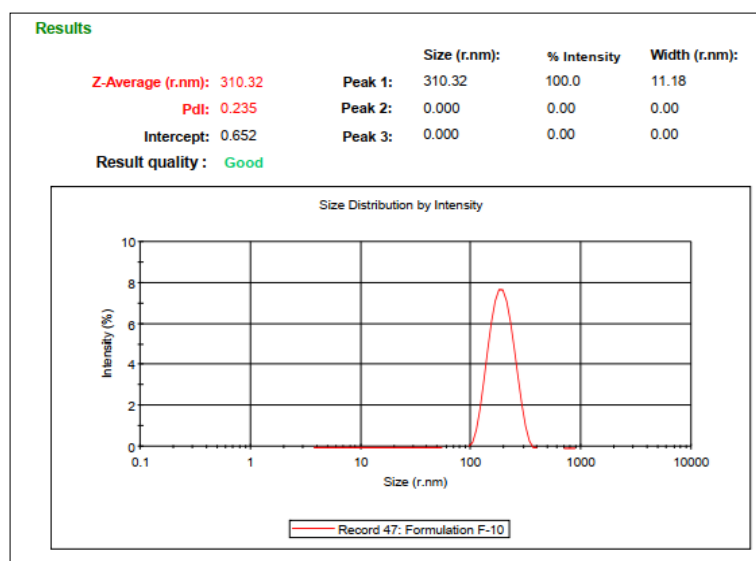


Figure 1: Graph of Particle size of optimized formulation F10

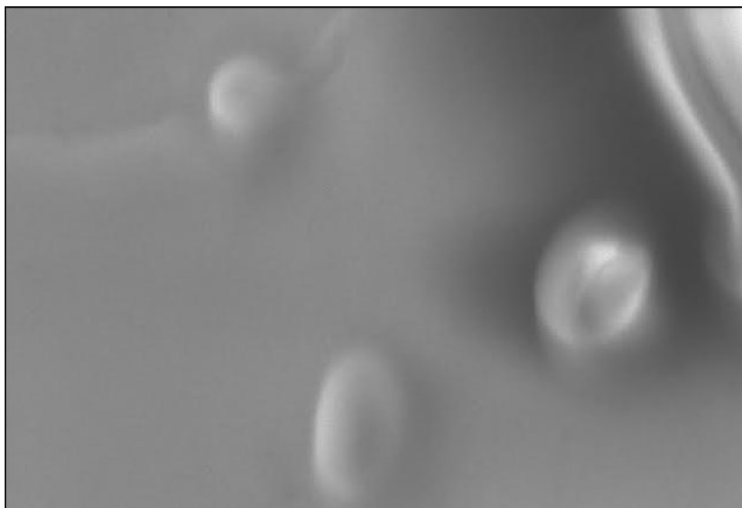


Figure 2: TEM image of phospholipids complex

Table 6: *In-vitro* drug release data for optimized formulation F10

Time (h)	Square Root of Time(h) ^{1/2}	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.23	1.306	79.77	1.902
1	1.000	0	31.12	1.493	68.88	1.838
2	1.414	0.301	52.23	1.718	47.77	1.679
4	2.000	0.602	69.98	1.845	30.02	1.477
6	2.449	0.778	82.23	1.915	17.77	1.250
8	2.828	0.903	86.65	1.938	13.35	1.125
12	3.464	1.079	98.85	1.995	1.15	0.061

Table 7: Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R ²	R ²	R ²	R ²
F10	0.8597	0.9433	0.9612	0.9719

CONCLUSION

The results indicate that the phospholipid complex of *Piper cubeba* extract is a promising approach for enhancing the bioavailability, stability, and controlled release of the plant's bioactive compounds. The optimized formulation (F10) with its favorable particle size, high entrapment efficiency, and sustained release profile could significantly improve the therapeutic potential of *Piper cubeba*, making it a viable candidate for drug delivery systems. Further studies, including in vivo testing and clinical evaluations, are required to fully understand the therapeutic benefits and safety of this formulation.

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