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FORMULATION AND CHARACTERIZATION OF PHOSPHOLIPID COMPLEX OF *PIPER CUBEBA*

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ABSTRACT

This study focuses on the formulation and characterization of phospholipid complexes of Piper cubeba to enhance the bioavailability of its bioactive constituents. Various formulations were optimized by varying the ratios of phospholipids and cholesterol, extract concentrations, and dichloromethane concentrations. The hydroalcoholic extract yielded a percentage of 10.5%, containing significant phytochemicals, including flavonoids and phenols. The optimized formulation demonstrated a particle size of 265.45 nm and an entrapment efficiency of 76.45%. *In vitro* dissolution studies revealed a cumulative drug release of 98.85% over 12 hours, with the release kinetics fitting the Korsmeyer-Peppas model (R^2 = 0.9535). These results indicate the potential of phospholipid complexes in enhancing the therapeutic efficacy of *Piper cubeba*, warranting further investigation into their clinical applications.

Keywords: *Piper cubeba*, Phospholipid complex, Bioavailability, Entrapment efficiency, *In vitro* drug release, Phytochemical screening, Formulation optimization.

INTRODUCTION

Piper cubeba, commonly known as cubeb pepper, is a tropical flowering vine belonging to the Piperaceae family. Traditionally used in various herbal medicine practices, it has been recognized for its potential therapeutic properties, including antimicrobial, anti-inflammatory, and analgesic effects (Mishra *et al.,* 2014). However, the clinical application of Piper cubeba is limited by its poor solubility and bioavailability, primarily due to its lipophilic nature. This necessitates the development of innovative formulation strategies to enhance its pharmacological efficacy.

Phospholipid complexes, particularly those utilizing phosphatidylcholine, have emerged as a promising approach to improve the solubility and absorption of poorly soluble drugs (Huang *et al.,* 2016). These complexes facilitate drug delivery by forming stable structures that can enhance cellular uptake and improve drug release profiles. By encapsulating Piper cubeba extracts within phospholipid matrices, the bioavailability of the active constituents can be significantly enhanced, making it a more viable therapeutic agent.

The aim of this study is to formulate and characterize a phospholipid complex of Piper cubeba, focusing on optimizing its physicochemical properties, evaluating its solubility enhancement, and assessing its potential for improved therapeutic outcomes.

Understanding the interaction between Piper cubeba components and phospholipids will provide insights into the formulation's efficacy and pave the way for its use in clinical applications.

MATERIALS AND METHODS

Materials

The study utilized various chemicals sourced from reputable suppliers. Key reagents included Potassium Mercuric Iodide**,** Iodine, and Potassium Iodide for analytical procedures. Picric Acid and Sodium Nitropruside served as indicators, while Sodium Hydroxide and Pyridine were used for pH adjustments. Other important materials included Ferric Chloride for complex formation, and solvents like Methanol**,** Ethanol, and Chloroform for extraction. These carefully selected materials were essential for the formulation and characterization of the phospholipid complex of *Piper cubeba*.

Methods

Collection of plant material

Seeds of *Piper cubeba* were collected from local area of Bhopal (M.P), India in the months of January, 2024.

Extraction by maceration process

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs (Mukherjee, 2007). 50 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:

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

Qualitative phytochemical tests

Phytochemical examinations were carried out extracts as per the following standard methods (Kokate, 1994).

Quantitative studies of bioactive constituents

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method (Parkhe and Bharti, 2019). 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 (Parkhe and Bharti, 2019). 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% $AlCl_3$ 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* extract 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 (Guo *et al.,* 2014).

Characterization of prepared phospholipids complex

Entrapment efficiency

Phospholipids complex preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4^0C (Yadav *et al.,* 2015). 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:

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

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 (Huang *et al.,* 2019).

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 phosphotungustic acid (Ruan *et al.,* 2010). 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) (Costa and Lobo, 2001). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37 ± 0.5^0C 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 (37^0C) was replaced every time with the same quantity of the sample and takes the absorbance at 256 nm using spectroscopy.

RESULTS AND DISCUSSION

The different formulations of the phospholipid complex were optimized for the ratio of phospholipids to cholesterol, extract concentration, and dichloromethane concentration (Table 1). The formulations aimed to enhance the encapsulation efficiency and stability of the active components derived from *Piper cubeba*.

The extraction process yielded a hydroalcoholic extract with a percentage yield of 10.5% (Table 2). Phytochemical screening (Table 3) indicated the presence of flavonoids, phenols, proteins, carbohydrates, saponins, diterpenes, and tannins, suggesting that the extract is rich in bioactive compounds.

The entrapment efficiency and particle size of various formulations were evaluated (Table 5). The optimized formulation F10 demonstrated the best performance with a particle size of 265.45 nm and an entrapment efficiency of 76.45%. This indicates a successful encapsulation of the bioactive constituents.

The *in vitro* drug release profile for formulation F10 showed a cumulative drug release of 98.85% over 12 hours (Table 6). The release data were analyzed using various models, with the Korsmeyer-Peppas model yielding the highest regression coefficient (R^2 = 0.9535), indicating a good fit for the release kinetics (Table 7).

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

Table 2: Result of percentage yield of extract

S. No.	Extract	Percentage yield (%)
1.	Hydroalcoholic	10.5%

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 Alkaline test	 +ve +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	0.55	0.84

Table 5: Particle size and entrapment efficiency of phospholipids complex

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	395.45	62.23
F2	369.41	65.45
F3	**355.25**	**69.98**
F4	375.45	64.13
F5	335.65	68.85
F6	**310.25**	**73.32**
F7	347.74	67.74
F8	320.12	66.32
F9	320.12	64.45
F10	**265.45**	**76.45**
F11	310.25	66.32
F12	316.58	71.12

Average of three determinations (n=3)

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	26.65	1.426	73.35	1.865
1	1	0	42.25	1.626	57.75	1.762
2	1.414	0.301	63.22	1.801	36.78	1.566
4	2	0.602	73.32	1.865	26.68	1.426
6	2.449	0.778	81.52	1.911	18.48	1.267
8	2.828	0.903	92.23	1.965	7.77	0.890
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	0.8296	0.9650	0.9389	0.9535

CONCLUSION

The study successfully formulated and characterized a phospholipid complex of Piper cubeba, demonstrating good entrapment efficiency and favorable release kinetics. These findings suggest the potential for using phospholipid complexes to enhance the therapeutic efficacy of natural extracts. Further studies are warranted to assess the bioavailability and clinical applications of this formulation.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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