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FORMULATION AND CHARACTERIZATION OF PHYTO-PHOSPHOLIPID COMPLEXES OF MEDICINAL PLANT EXTRACT OF *OPUNTIA FICUS-INDICA*

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ABSTRACT

The study aimed to formulate and characterize phyto-phospholipid complexes of *Opuntia ficus-indica* extract to enhance its bioavailability and therapeutic efficacy. The hydroalcoholic extract of *Opuntia ficus-indica* yielded 13.8% and was subjected to phytochemical screening, revealing the presence of glycosides, phenols, proteins, saponins, and tannins. Total phenol and flavonoid contents were quantified as 0.765 mg/100 mg and 0.841 mg/100 mg, respectively. Various formulations of phyto-phospholipid complexes were prepared and evaluated for particle size and entrapment efficiency. The optimized formulation, F10, demonstrated a particle size of 310.32 nm and an entrapment efficiency of 74.65%. *In-vitro* drug release studies indicated a sustained release profile, with the Korsmeyer-Peppas model providing the best fit (R^2 = 0.956). These results suggest that phyto-phospholipid complexes significantly improve the stability and bioavailability of *Opuntia ficus-indica* extracts, highlighting their potential for enhanced therapeutic applications.

Keywords: *Opuntia ficus-indica*, phyto-phospholipid complex, phytosome, bioavailability, hydroalcoholic extract, phytochemical screening.

INTRODUCTION

The use of medicinal plants in modern therapeutics has gained significant attention due to their therapeutic potential and minimal side effects. Among these plants, *Opuntia ficus-indica*, commonly known as prickly pear cactus, has shown promising pharmacological properties, including antioxidant, anti-inflammatory, and anticancer activities (Habibi *et al.,* 2020). However, the bioavailability of plant extracts often limits their therapeutic efficacy. To overcome this challenge, the formulation of phyto-phospholipid complexes, also known as phytosomes, has been explored. These complexes enhance the solubility, stability, and bioavailability of bioactive compounds from plant extracts, thereby improving their therapeutic potential (Agarwal *et al.,* 2012).

Opuntia ficus-indica is rich in bioactive compounds such as flavonoids, polyphenols, and betalains, which contribute to its medicinal properties (Galati *et al.,* 2003). However, the poor water solubility and stability of these compounds necessitate the development of novel delivery systems. Phytosomes represent an innovative approach to enhance the bioavailability of these phytoconstituents. The complexation of plant extracts with phospholipids forms a lipid-compatible molecular complex, facilitating better absorption and utilization of the bioactive compounds (Maiti *et al.,* 2006).

The objective of this study is to formulate and characterize phyto-phospholipid complexes of *Opuntia ficus-indica* extract. The study will evaluate the physical and chemical properties of the complexes, including their solubility, stability, and in vitro release profile. By enhancing the bioavailability of the bioactive compounds in *Opuntia ficus-indica*, these phyto-phospholipid complexes have the potential to improve the therapeutic efficacy of this medicinal plant.

MATERIALS AND METHODS

Material

Various chemicals were used in this study, sourced from reputable suppliers. Potassium mercuric iodide, picric acid, and ferric chloride were obtained from Thomas Baker, Mumbai. Iodine, potassium iodide, sodium nitroprusside, sodium hydroxide, Folin-Ciocalteu reagent, and lead acetate were supplied by Loba Chemie Pvt. Ltd., Mumbai. Potassium bismuth iodide, pyridine, gelatin, nitric acid, copper acetate, and sodium chloride came from S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and chloroform were provided by Qualigens Fine Chemicals, Mumbai. Fehling's solution was sourced from Central Drug House Ltd., New Delhi.

Extraction by maceration process

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs (Mukherjee; 2007). 65 gram plant materials of *Opuntiaficus-indica* 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 *Opuntiaficus-indica* were extracted with hydroalcoholic solvent (ethanol: water: 65:35 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 *Opuntia ficus-indica* 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

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 (Gnananath *et al.,* 2017).

Entrapment efficiency

Phospholipids complex preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4^0C. The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped *Opuntiaficus-indica* 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 *Opuntiaficus-indica* in 1 ml dispersion (Yadav *et al.,* 2015). 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).

***In vitro* dissolution rate studies**

In vitro drug release of the sample was carried out using USP- type II dissolution apparatus (Paddle type) [100]. 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 256.0 nm using spectroscopy (Ruan *et al.,* 2010).

RESULTS AND DISCUSSION

The hydroalcoholic extract of *Opuntia ficus-indica* resulted in a percentage yield of 13.8% (Table 2). The phytochemical screening revealed the presence of various bioactive constituents (Table 3). Glycosides, phenols, proteins, saponins, and tannins were present in the hydroalcoholic extract, while alkaloids, carbohydrates, flavonoids (in the alkaline test), and diterpenes were absent. This diverse phytochemical profile supports the potential therapeutic benefits of *Opuntia ficus-indica*.

The quantification of total bioactive constituents showed that the hydroalcoholic extract contained 0.765 mg/100 mg of total phenols and 0.841 mg/100 mg of total flavonoids (Table 4). These compounds are known for their antioxidant and anti-inflammatory properties, which contribute to the medicinal value of the extract.

The particle size and entrapment efficiency of the phyto-phospholipid complexes varied across different formulations (Table 5). The optimized formulation, F10, had the smallest particle size of 310.32 nm and the highest entrapment efficiency of 74.65%. This indicates that F10 was the most effective in encapsulating the bioactive compounds, ensuring better stability and bioavailability.

The *in-vitro* drug release study for the optimized formulation F10 demonstrated a sustained release profile (Table 6). The cumulative percentage of drug release increased progressively, reaching 90.25% at 12 hours. The log-transformed data showed a linear relationship, indicating a controlled release mechanism.

Regression analysis of the drug release data (Table 7) showed that the release kinetics of the optimized formulation F10 best fit the Korsmeyer-Peppas model (R^2 = 0.956), followed by the Higuchi model (R^2 = 0.943). This suggests that the drug release from the phyto-phospholipid complex was governed by a combination of diffusion and erosion mechanisms.

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	13.8%

Table 3: Result of phytochemical screening of *Opuntiaficus-indica* 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 *Opuntiaficus-indica*

S. No.	Extract	Total phenol (mg/100mg)	Total Flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.765	0.841

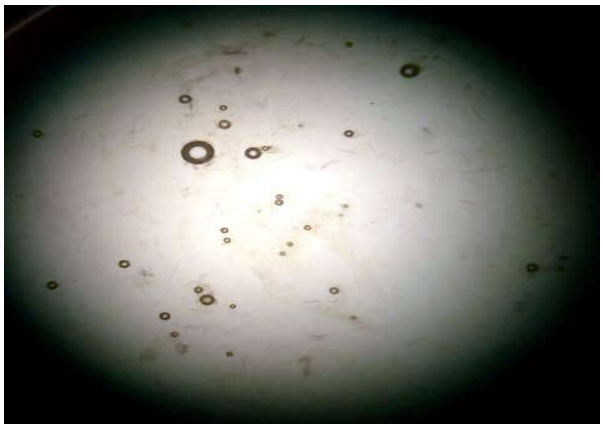

Figure 1: Microscopic observation of optimized batch F10

Table 5: Particle size and entrapment efficiency of phospholipids complex

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	385.45	55.85
F2	362.25	62.23
F3	347.85	62.23
F4	358.85	63.32
F5	347.74	65.74
F6	321.12	70.25
F7	345.78	67.85
F8	326.65	64.78
F9	325.65	61.25
F10	310.32	74.65
F11	314.56	65.45
F12	345.65	68.87

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	18.85	1.275	81.15	1.909
1	1	0	34.45	1.537	65.55	1.817
2	1.414	0.301	49.98	1.699	50.02	1.699
4	2	0.602	65.58	1.817	34.42	1.537
6	2.449	0.778	79.98	1.903	20.02	1.301
8	2.828	0.903	85.65	1.933	14.35	1.157
12	3.464	1.079	90.25	1.955	9.75	0.989

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	0.828	0.683	0.943	0.956

CONCLUSION

The study successfully formulated and characterized phyto-phospholipid complexes of *Opuntia ficus-indica* extract. The optimized formulation F10 exhibited excellent particle size, entrapment efficiency, and a sustained drug release profile. These findings support the potential of phyto-phospholipid complexes to enhance the bioavailability and therapeutic efficacy of *Opuntia ficus-indica* extracts. Further in-vivo studies are warranted to validate these results and explore the clinical applications of these complexes.

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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