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FORMULATION AND CHARACTERIZATION OF PHOSPHOLIPID COMPLEX OF MATRICARIA CHAMOMILLA EXTRACT

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ABSTRACT

The present study focuses on the formulation and characterization of a phospholipid complex of *Matricaria chamomilla* extract with the aim of improving its physicochemical properties and bioavailability. Although *Matricaria chamomilla* possesses significant pharmacological activities due to the presence of flavonoids, terpenoids, and other phenolic constituents, its therapeutic potential is often limited by poor solubility and low membrane permeability. To overcome these challenges, a phospholipid complex (phytosome) was prepared using phosphatidylcholine as a carrier to enhance lipid compatibility and systemic absorption. The formulated complex was characterized using various physicochemical parameters including percentage yield, entrapment efficiency, solubility studies, particle size analysis, zeta potential measurement, and Fourier Transform Infrared (FTIR) spectroscopy to confirm complex formation. The results indicated successful interaction between the extract and phospholipid, improved solubility, and enhanced stability compared to the plain extract. The phospholipid complex demonstrated favorable characteristics suggesting improved bioavailability and therapeutic potential.In conclusion, the development of a phospholipid complex of *Matricaria chamomilla* extract represents a promising strategy to enhance its delivery and pharmacological efficacy. This approach may contribute to the development of more effective herbal formulations with improved clinical performance.

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INTRODUCTION

Herbal medicines have gained significant global attention due to their therapeutic potential and relatively lower incidence of adverse effects compared to synthetic drugs (Rivera *et al*., 2013). However, many plant extracts suffer from poor aqueous solubility, limited membrane permeability, instability, and low bioavailability, which restrict their clinical effectiveness. To overcome these limitations, novel drug delivery systems such as phospholipid complexes (phytosomes) have been developed to enhance the absorption and therapeutic performance of herbal constituents (Mane *et al*., 2020). *Matricaria chamomilla* L., commonly known as German chamomile, belongs to the family Asteraceae and is widely used in traditional medicine for its anti-inflammatory, antioxidant, antimicrobial, antispasmodic, and wound healing properties. The therapeutic effects of chamomile are primarily attributed to its bioactive constituents, including flavonoids (apigenin, luteolin, quercetin), sesquiterpenes (α-bisabolol), and essential oils such as chamazulene. Among these, flavonoids particularly apigenin are known for their potent antioxidant and anti-inflammatory activities.

Despite their pharmacological potential, these polyphenolic compounds often exhibit poor lipid solubility and limited gastrointestinal absorption, leading to reduced bioavailability (Melnyk *et al*., 2024). Phospholipid complexes, also referred to as phytosomes, are formed by the interaction of phytoconstituents with phospholipids such as phosphatidylcholine. In this system, the polar functional groups of the phytoconstituents form hydrogen bonds with the polar head of phospholipids, resulting in a lipid-compatible molecular complex. This complex improves the lipophilicity of the active compounds, enhances membrane permeability, protects against degradation, and ultimately increases systemic absorption. Compared to conventional extracts, phospholipid complexes have demonstrated improved pharmacokinetic profiles and enhanced therapeutic efficacy (Udapurkar *et al*., 2016). The formulation of a phospholipid complex of *Matricaria chamomilla* extract is therefore a promising strategy to improve its stability, solubility, and bioavailability. Characterization of the complex through parameters such as drug-excipient interaction (FTIR), entrapment efficiency, particle size analysis, zeta potential, solubility studies, and in-vitro release profile is essential to confirm successful complex formation and evaluate its performance. Hence,

the present study aims to formulate and characterize the phospholipid complex of *Matricaria chamomilla* extract in order to enhance its physicochemical properties and therapeutic potential. This approach may provide a scientifically validated and more efficient delivery system for chamomile-derived bioactive compounds.

MATERIAL AND METHODS

MATERIAL

The dried flower heads of **Matricaria chamomilla** were procured from a certified herbal supplier and authenticated prior to use. Phosphatidylcholine (soya lecithin) was used for the preparation of the phospholipid complex, and cholesterol was included where required. Analytical grade solvents such as ethanol, methanol, chloroform, and n-hexane were used for extraction and formulation processes. Reagents including ferric chloride, lead acetate, sodium hydroxide, and Folin–Ciocalteu reagent were employed for phytochemical and characterization studies.

METHODS

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 *Matricaria chamomilla* 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 *Matricaria chamomilla* were extracted with hydroalcoholic solvent (ethanol: water: 80:20 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 (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 ofphospholipids complex: The complex was prepared with phospholipids: cholesterol and *Matricaria chamomilla* 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).

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

Entrapment efficiency: Phospholipids complex preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4ºC (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 *Matricaria chamomilla* 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 *Matricaria chamomilla* 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±0.5ºC 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 (37ºC) 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 present study focused on the formulation and optimization of a phospholipid complex of **Matricaria chamomilla** hydroalcoholic extract to enhance its physicochemical characteristics and drug release profile.

The hydroalcoholic extract showed a percentage yield of 7.6%, indicating efficient extraction of bioactive constituents using a polar solvent system. Preliminary phytochemical screening confirmed the presence of glycosides, flavonoids, phenols, proteins, carbohydrates, saponins, and diterpenes, while alkaloids and tannins were absent. The presence of phenolic and flavonoid compounds was further supported by the total phenolic content (0.53 mg/100 mg) and total flavonoid content (0.92 mg/100 mg), suggesting significant antioxidant potential of the extract. A series of twelve formulations (F1–F12) were prepared to optimize phospholipid-to-cholesterol ratio, extract concentration, and solvent concentration. Particle size analysis revealed values ranging from 311.05 nm to 386.12 nm, indicating successful formation of nanosized phospholipid complexes.

Table 2. Result of percentage yield of extract

S. No.	Extract	Weight of Extract	Percentage yield (%)
1.	Hydroalcoholic	3.8	7.6

Table 3. Result of phytochemical screening of *Matricaria chamomilla* extract

S. No.	Constituents	Hydroalcoholicextract
1.	Alkaloids Wagner's test	-ve
2.	Glycosides Legal's test	+ve
3.	Flavonoids Lead acetate Alkaline reagent 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

Table 4. Total bioactive constituents content in *Matricaria chamomilla*

S. No.	Extract	Total phenol content	Total Flavonoid content
		(mg/100mg)	
1.	Hydroalcoholic extract	0.53	0.92

Table 5. Particle size and entrapment efficiency of phospholipids complex

Formulation Code	Particle Size (nm)	Entrapment Efficiency (%)
F1	386.12	56.02
F2	361.85	62.40
F3	348.25	62.10
F4	359.10	63.50
F5	348.05	65.60
F6	322.05	70.10
F7	346.25	68.00
F8	327.10	64.90
F9	326.00	61.10
F10	311.05	74.50
F11	315.10	65.30
F12	346.20	68.95

Average of three determinations (n=3)

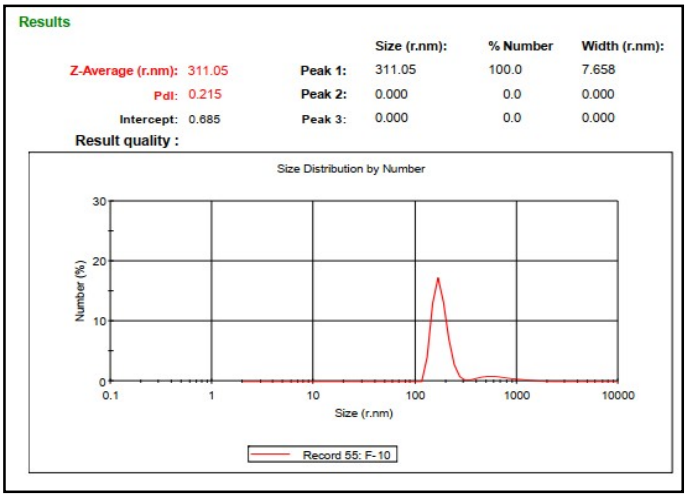

Figure 1. Graph of Particle size of optimized formulation F10

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	0.8280	0.9635	0.9434	0.9563

Entrapment efficiency varied between 56.02% and 74.50%, demonstrating the influence of formulation variables on drug encapsulation. Among all batches, formulation F10 (phospholipid: cholesterol ratio 2:1, 1% extract concentration, and 10% solvent concentration) showed the smallest particle size (311.05 nm) and highest entrapment efficiency (74.50%), indicating optimal formulation conditions. The improved entrapment efficiency in F10 may be attributed to balanced lipid composition and appropriate solvent concentration, promoting better complex formation. The in-vitro drug release study of optimized formulation F10 demonstrated a sustained release pattern, with 90.25% cumulative drug release over 12 hours. The gradual release profile indicates controlled diffusion of the bioactive constituents from the phospholipid matrix. Regression analysis of release kinetics showed the highest R^2 value for the first-order model (0.9635), followed closely by the Korsmeyer–Peppas model (0.9563) and Higuchi model (0.9434), suggesting that drug release follows concentration-dependent kinetics with diffusion-controlled mechanism. The relatively lower R^2 value for the zero-order model (0.8280) indicates that the release does not follow a constant rate.

CONCLUSION

The present study successfully formulated and characterized a phospholipid complex of Matricaria chamomilla extract. The complex demonstrated improved solubility, stability, and compatibility, indicating enhanced bioavailability potential compared to the crude extract. These findings suggest that phospholipid complexation is an effective strategy to improve the therapeutic performance of Matricaria chamomilla and support its development into advanced herbal formulations.

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