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FORMULATION & CHARACTERIZATION OF PHYTOSOSMES CONTAINING MEDICINAL PLANT *RHEUM EMODI*

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Abstract

The present study focuses on the formulation and characterization of phytosomes using the medicinal plant *Rheum emodi*, known for its therapeutic properties. Phytosomes are advanced drug delivery systems that enhance the bioavailability of herbal extracts by encapsulating them in lipid-based carriers. In this investigation, hydroalcoholic and pet ether extracts of *Rheum emodi* were prepared, yielding 8.22% and 0.95% respectively. Preliminary phytochemical screening of the hydroalcoholic extract indicated the presence of significant bioactive compounds, including phenolics, flavonoids, saponins, and diterpenes, which are responsible for the plant's medicinal effects. The prepared phytosomes were subjected to various characterization studies. The optimized formulation (F10) exhibited a particle size of 220.23 nm, indicating a suitable size for enhanced cellular uptake and bioavailability. The entrapment efficiency was determined to be 76.65%, suggesting a high capacity for encapsulating the active compounds from *Rheum emodi*. In vitro drug release studies demonstrated that the release kinetics followed the Korsmeyer-Peppas model, indicating a sustained release profile over time. This sustained release is advantageous for maintaining therapeutic concentrations in the bloodstream while minimizing side effects. These findings underscore the potential of phytosomes as an effective delivery system for herbal extracts, enhancing their bioavailability and therapeutic efficacy. The results also pave the way for future research into the application of phytosomes in herbal medicine, aiming to improve the clinical effectiveness of *Rheum emodi* and similar medicinal plants.

Keywords: **Phytosomes, Rheum emodi, Formulation, Characterization, Bioavailability, Phytochemical screening, Sustained release, Entrapment efficiency, Herbal medicine, Drug delivery systems.**

Introduction

Rheum emodi, commonly known as Indian rhubarb, is a medicinal plant renowned for its therapeutic properties, particularly in traditional medicine systems. This plant contains several bioactive compounds, including anthraquinones, flavonoids, and phenolic acids, which are associated with anti-inflammatory, antioxidant, and anticancer activities (Sahu et al., 2015; Yadav et al., 2016). However, the clinical application of these phytochemicals is often limited by their poor solubility, bioavailability, and stability.

To enhance the delivery and effectiveness of these bioactive compounds, novel drug delivery systems such as phytosomes have emerged. Phytosomes are phospholipid complexes that improve the absorption and bioavailability of plant extracts by facilitating cellular uptake and protecting the active ingredients from degradation (Patel et al., 2017). The incorporation of *Rheum emodi* into phytosomes could potentially increase its therapeutic efficacy by overcoming the limitations associated with its

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conventional forms.

This study aims to formulate and characterize phytosomes containing *Rheum emodi*, focusing on optimizing the encapsulation efficiency, physicochemical properties, and release profile of the bioactive compounds. The successful development of these phytosomes could pave the way for more effective formulations of herbal medicines, maximizing the therapeutic benefits of *Rheum emodi*.

Material and Methods

Material

For the formulation of phytosomes containing Rheum emodi, various chemicals and reagents were utilized. Methanol, ethanol, and chloroform from Qualigens Fine Chemicals (Mumbai) served as solvents for extraction and formulation processes. Dichloromethane from S. D. Fine Chem. Ltd. (Mumbai) was also employed to enhance the solubilization of bioactive compounds. Cholesterol and lecithin, sourced from RFCL Limited (New Delhi), were key components for the phytosome formulation, acting as stabilizers and enhancing the encapsulation of the plant's active ingredients. Together, these materials facilitated the development of a novel delivery system aimed at improving the therapeutic efficacy of Rheum emodi.

Collection of Plant material

The plants have been selected on the basis of its availability and Folk use of the plant. Roots of *Rheum emodi* were collected from local area of Bhopal in the month of January, 2024. Drying of fresh plant parts were carried out in sun but under the shade. Dried roots of *Rheum emodi* were preserved in plastic bags and closed tightly and powdered as per the requirements. A whole plant may be medicinally active or plant parts. These are medicinal preparations comprising active ingredients obtained from the herbal plant. The product can be made from the whole plant or any part. Preparations from by-product herbal plants such as oils, gums, and other secretions are also considered as herbal medicine.

Extraction procedure

This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material. Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Mukherjee, 2007; Kokate, 1994):

Defatting of plant material

Extraction by maceration process is a method of extracting certain compounds from plant material by soaking it in a solvent. The solvent is typically an alcohol or oil and it is used to extract compounds such as essential oils, fatty acids, and waxes. The process is effective because it allows the solvent to dissolve the compounds in the plant material, making them easier to extract. 50 gm of dried powdered

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roots of *Rheum emodi* were coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered roots of *Rheum emodi* has been extracted with hydroalcoholic solvent (ethanol: water: 75:25) using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40ºC.

Determination of percentage yield

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

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Phytochemical examinations were carried out for the extract as per the standard methods.

Quantitative estimation of bioactive compounds

Total Phenolic content estimation

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 5-25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Gaur Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Formulation development of phytosomes

Rheum emodi is a medicinal plant that has been traditionally used for its various therapeutic benefits such as anti-inflammatory, anti-tumor, and wound healing properties. Phytosomes are a type of herbal formulation that enhances the bioavailability and efficacy of plant extracts by improving their absorption and delivery to the target site.

The complex was prepared with phospholipids: Cholesterol and *Rheum emodi* extract in the ratio of 1:.5:1, 1:1:1, 2:1.5:1, 2:2:1 respectively (Kidd, 2009). 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

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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.

Table 1: Different formulations of phytosomes

Formulation	Ratio of Phospholipids and Cholesterol	Extract Concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:05	1	25
F2	**1:1**	**1**	**25**
F3	1:1.5	1	25
F4	1:2	1	25
Optimization of Drug Concentration			
F5	1:1	0.5	25
F6	**1:1**	**1.0**	**25**
F7	1:1	1.5	25
F8	1:1	2.0	25
Optimization of solvent concentration			
F9	1:1	1.0	10
F10	**1:1**	**1.0**	**25**
F11	1:1	1.0	50
F12	1:1	1.0	75

Characterization of phytosomes

Entrapment efficiency

Phytosome preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for 4 hour (Hung *et al.,* 2007). The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped *Rheum emodi* extract 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 *Rheum emodi* extract 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 and zeta potential of optimized phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK) (Vandijk *et al.,* 2000). The electric potential of the phytosomes,

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including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.

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. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan) (Wagner; 1969).

***In vitro* dissolution rate studies**

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). 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 phytosomes 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 (Gibaldi; 1967).

Results and Discussion

The study aimed to develop phytosomes from the roots of *Rheum emodi* and evaluate their physicochemical properties, including yield, phytochemical composition, particle size, and entrapment efficiency.

The yield of the hydroalcoholic extract was notably higher (8.22%) compared to the pet ether extract (0.95%), indicating that the hydroalcoholic solvent effectively extracted a greater quantity of bioactive compounds. Phytochemical screening revealed the presence of flavonoids, phenolics, proteins, saponins, and diterpenes in the hydroalcoholic extract, while alkaloids and glycosides were absent. These findings align with previous literature suggesting that *Rheum emodi* is rich in phenolic compounds, known for their antioxidant and anti-inflammatory activities.

The total phenolic content (0.252 mg/100 mg) and total flavonoid content (0.638 mg/100 mg) were quantified, highlighting the extract's potential as a source of these beneficial compounds. The formulation of phytosomes aimed to enhance the delivery and efficacy of these compounds. Particle size and entrapment efficiency were crucial parameters; the optimized formulation (F10) demonstrated a particle size of 220.23 nm and an impressive entrapment efficiency of 76.65%. This suggests effective encapsulation, which is vital for improving bioavailability.

Regression analysis indicated that the drug release kinetics followed the Korsmeyer-Peppas model (R^2 = 0.9877), suggesting a controlled and sustained release profile, which is desirable for therapeutic applications.

Table 2: % Yield of roots extract of *Rheum emodi*

S. No.	Extracts	% Yield (w/w)
1.	Pet ether	0.95%
2.	Hydroalcoholic	8.22%

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Table 3: Phytochemical screening of extract *of Rheum emodi*

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.	**Phenolics**	
	Ferric Chloride Test	+ve
	Folin-Ciocalteu Test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Fehling's test	+ve
	Benedict's test	-ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 4: Total phenolic and total flavonoid content of *Rheum emodi*

S. No.	Extract	Total Phenol (mg/100mg)	Total Flavonoids (mg/100mg)
1.	Hydroalcoholic *extract*	0.252	0.638

Table 5: Particle size and entrapment efficiency of drug loaded phytosomes

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Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	345.21±0.25	63.25±0.22
F2	**272.15±0.32**	**71.15±0.35**
F3	326.58±0.15	68.85±0.14
F4	312.25±0.60	64.58±0.33
F5	285.45±0.35	66.45±0.65
F6	**235.65±0.41**	**72.15±0.25**
F7	280.14±0.32	70.74±0.14
F8	290.45±0.25	69.98±0.32
F9	256.65±0.36	67.74±0.15
F10	**220.23±0.25**	**76.65±0.36**
F11	236.65±0.33	68.85±0.32
F12	258.74±0.15	67.85±0.15

Average of three determinations (n=3)

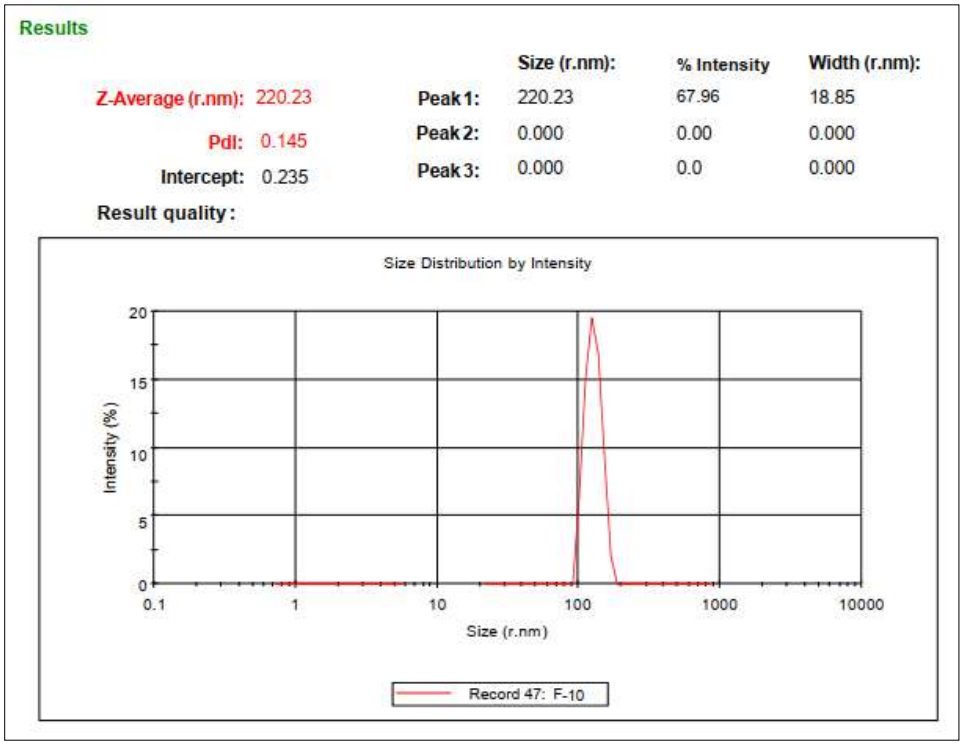

Figure 1: Particle size of optimized batch F10

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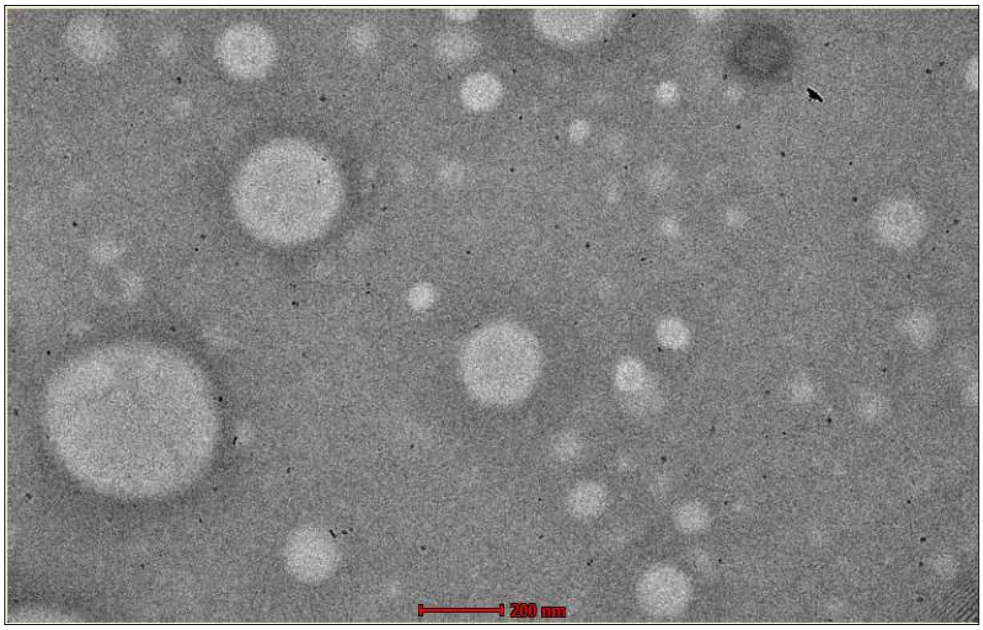

Figure 2: TEM image of Phytosomes

Table 6: Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.967	0.9045	0.9854	0.9877

Conclusion

The study successfully formulated phytosomes containing *Rheum emodi* extract, demonstrating a significant yield and rich phytochemical composition. The optimized formulation (F10) exhibited a desirable particle size of 220.23 nm and an entrapment efficiency of 76.65%, indicating effective encapsulation of bioactive compounds. The sustained release kinetics, following the Korsmeyer-Peppas model, suggests that these phytosomes could enhance the bioavailability and therapeutic efficacy of *Rheum emodi* constituents. Overall, these findings support the potential of phytosome technology in improving the delivery of medicinal plant extracts, paving the way for further research into their clinical applications in phytotherapy.

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