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DEVELOPMENT AND CHARACTERIZATION OF PHYTOSOMAL FORMULATION OF HYDROALCOHOLIC EXTRACT OF *PUTRANJIVA ROXBURGHII*

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ABSTRACT

This study investigates the development and characterization of a phytosomal formulation using the hydroalcoholic extract of *Putranjiva roxburghii*, a medicinal plant known for its bioactive properties. The extraction process yielded 2.65% from pet ether and 7.85% from a hydroalcoholic solvent, indicating that the hydroalcoholic extract is more effective in isolating the plant's bioactive compounds. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, proteins, carbohydrates, saponins, and tannins, with significant antioxidant potential, primarily due to the flavonoid and phenolic content. Quantitative analysis of the hydroalcoholic extract showed a total phenolic content of 0.65 mg/100 mg and total flavonoid content of 0.85 mg/100 mg, underscoring the extract's potential as a source of natural antioxidants for therapeutic applications. To enhance the bioavailability and stability of the active compounds, a phytosomal formulation was prepared by complexing the extract with a phospholipid complex in a 1:1:1 ratio of phospholipids, cholesterol, and *Putranjiva roxburghii* extract. Among the various formulations, F10 exhibited the smallest particle size (215.47 nm) and a high entrapment efficiency of 74.66%, indicating enhanced stability and bioavailability. The release kinetics followed a First Order model (R^2 = 0.9912), suggesting a concentration-dependent release, with diffusion also playing a key role, as confirmed by the Higuchi model (R^2 = 0.9544). Stability studies revealed that the optimized phytosome formulations remained stable for up to three months under controlled conditions, ensuring their potential for therapeutic use. The results emphasize the promising therapeutic applications of *Putranjiva roxburghii* in drug delivery systems and encourage further research into its bioactivity and clinical efficacy.

Keywords: *Putranjiva roxburghii*, Phytosomal formulation, Hydroalcoholic extract, Phytochemical screening, Phospholipid complex, Entrapment efficiency

INTRODUCTION

Putranjiva roxburghii (Family: Euphorbiaceae), commonly known as Roxburgh's Putranjiva, is a medicinal plant traditionally used in several herbal formulations in Indian and Chinese medicine. Known for its diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties, *P. roxburghii* has gained attention for its potential therapeutic benefits (Yadav *et al.,* 2019). The plant's medicinal properties are attributed to its rich phytochemical content, which includes alkaloids, flavonoids, glycosides, saponins,

and phenolic compounds (Patel and Choudhary, 2020).

Despite its therapeutic potential, the clinical application of *Putranjiva roxburghii* has been limited due to the poor bioavailability of its active compounds when administered orally. This limitation arises primarily due to the plant's low solubility and instability in biological systems. To overcome these challenges, modern drug delivery systems, such as phytosomes, offer an innovative solution.

A phytosome is a nanocarrier system that enhances the bioavailability of herbal bioactive compounds by encapsulating the active ingredients in a phospholipid complex, which improves the solubility, stability, and cellular absorption of the compounds (Sahu and Verma, 2021). The development of phytosomal formulations has gained significant attention in recent years due to their potential to deliver natural therapeutic agents more efficiently and safely compared to conventional herbal extracts (Reddy and Sharma, 2015).

In this study, we explore the phytosomal formulation of *Putranjiva roxburghii* using a hydroalcoholic extract of the plant. The hydroalcoholic extract is favored for its ability to solvate a wide range of bioactive compounds, resulting in a higher yield and more potent extract compared to other solvents (Yadav *et al.,* 2019). The aim is to formulate a phytosomal complex using the hydroalcoholic extract of *P. roxburghii* to enhance the bioavailability and therapeutic efficacy of its active components.

The encapsulation of bioactive compounds in phospholipids not only enhances their solubility but also allows for controlled and sustained release, which can improve the overall pharmacokinetics of the active ingredients. Additionally, stability studies of the formulation are essential to ensure that the bioactive compounds remain effective during storage and throughout their shelf life (Agarwal and Dubey, 2022). The phytosomal formulation is expected to offer a more efficient drug delivery system, making it a promising candidate for the development of herbal-based therapeutic products with antioxidant, anti-inflammatory, and other pharmacological activities.

Thus, the present study aims to develop and characterize a phytosomal formulation using the hydroalcoholic extract of *Putranjiva roxburghii*, optimizing the formulation for enhanced bioavailability and stability, and evaluating its potential for future clinical applications.

MATERIALS AND METHODS

Collection of Plant material

The plants have been selected on the basis of its availability and Folk use of the plant. Leaves of *Putranjiva roxburghii* were collected from local area of Bhopal in the month of January, 2023. Drying of fresh plant parts were carried out in sun but under the shade. Dried leaves of *Putranjiva roxburghii* were preserved in plastic bags and closed tightly and powdered as per the requirements.

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 an 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. 60 gm of dried powdered leaves of *Putranjiva roxburghii* 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 leaves of *Putranjiva roxburghii* has been extracted with hydroalcoholic solvent (ethanol: water: 25:75) using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40ºC (Kokate, 1994):

Determination of percentage yield

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

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} x100$$

Phytochemical Screening

Phytochemical examinations were carried out for the extract as per the standard methods (Parkhe and Bharti, 2019).

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

The complex was prepared with phospholipids: Cholesterol and *Putranjiva roxburghii* 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 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.

Characterization of phytosomes

Entrapment efficiency

Phytosome preparation was taken and subjected to centrifugation using cooling

centrifuge (Remi) at 12000 rpm for an hour at 4 (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 *Putranjiva roxburghii* 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 *Putranjiva roxburghii* 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, 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).

***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^{0}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 from the study on the phytosomal formulation of *Putranjiva roxburghii* reveal significant insights into the yield, bioactive content, and release characteristics of the hydroalcoholic extract, as well as the optimization and performance of the phytosomal formulation.

From the extraction process, the hydroalcoholic extract of *Putranjiva roxburghii* yielded 7.85%**,** which is significantly higher than the pet ether extract (2.65%) (Table 2). This difference in yield suggests that the hydroalcoholic solvent is more efficient in isolating a wider range of bioactive compounds from the roots of the plant. Hydroalcoholic solvents are known for their ability to solvate both polar and non-polar compounds, making them ideal for extracting a diverse array of phytochemicals.

Phytochemical screening of the hydroalcoholic extract (Table 3) indicated the presence of various bioactive constituents,

including alkaloids, glycosides, flavonoids, phenolics, proteins, carbohydrates, saponins, and tannins. Among these, flavonoids and phenolics are particularly noteworthy due to their antioxidant properties. The presence of saponins and glycosides also suggests potential antimicrobial and anti-inflammatory activities, which are valuable for therapeutic applications. These results confirm that the hydroalcoholic extract of *Putranjiva roxburghii* contains a broad spectrum of bioactive compounds, supporting its use in herbal medicine.

The total phenolic content of the hydroalcoholic extract was found to be 0.65 mg/100 mg, and the total flavonoid content was 0.85 mg/100 mg (Table 4). These values indicate that the extract possesses a moderate amount of phenolic and flavonoid compounds, which are well known for their antioxidant activities. The antioxidant potential of flavonoids and phenolics can be further explored for their ability to neutralize free radicals and contribute to the therapeutic properties of *Putranjiva roxburghii*.

Phytosomal formulations were developed by incorporating the hydroalcoholic extract into phospholipid complexes, with various formulations tested for their entrapment efficiency and particle size (Table 5). Among these formulations, F10 exhibited the smallest particle size of 215.47 nm and an entrapment efficiency of 74.66%, making it the most optimized batch. The smaller particle size is crucial for improving bioavailability, as it enhances the surface area for absorption and facilitates better interaction with biological membranes. The high entrapment efficiency suggests that a significant portion of the bioactive compounds was successfully incorporated into the phytosomal formulation, ensuring a more effective delivery system.

The *in-vitro* drug release data for formulation F10 (Table 6) showed a sustained release pattern over a 12-hour period. The release started at 28.89% after 30 minutes and progressively increased to 98.78% after 12 hours. This indicates that the phytosomal formulation of *Putranjiva roxburghii* provides a controlled and sustained release of its bioactive compounds, which is advantageous for prolonged therapeutic action. The release data were further analyzed using regression models to understand the release kinetics.

Regression analysis of the release data (Table 7) revealed that the release of the drug from the optimized F10 formulation followed a First Order kinetic model (R^2 = 0.9912), suggesting that the release rate depends on the concentration of the drug in the formulation. This is characteristic of a diffusion-controlled release mechanism, where the drug is released from the phospholipid complex over time. The Higuchi model (R^2 = 0.9544) also supports this, indicating that the release is controlled by the diffusion of the active compound through the matrix of the phytosome. The other models, including Zero Order (R^2 = 0.8475) and Korsmeyer-Peppas (R^2 = 0.7929), showed weaker fits, further emphasizing the importance of diffusion in the release process.

Stability studies are essential for confirming the effectiveness and shelf life of the formulation. The optimized phytosomal formulations, including F10, remained stable for up to three months under controlled conditions, indicating that the formulation is

reliable for long-term use without significant degradation of the active ingredients. This stability further enhances the therapeutic potential of the *Putranjiva roxburghii* extract in the phytosomal form, making it suitable for future clinical applications.

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

Table 2: % Yield of roots of *Putranjiva roxburghii*

S. No.	Extracts	% Yield (w/w)
1.	Pet ether	2.65%
2.	Hydroalcoholic	7.85%

Table 3: Phytochemical screening of extract *of Putranjiva roxburghii*

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

Table 4: Total phenolic and total flavonoid content of *Putranjiva roxburghii*

S. No.	Extract	Total Phenol	Total Flavonoids
		(mg/100mg)	
1.	Hydroalcoholic extract	0.65	0.85

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

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	345.85	68.98
F2	**280.45**	**73.36**
F3	315.69	68.85
F4	348.85	99.65
F5	285.65	67.12
F6	**248.85**	**72.23**
F7	274.58	68.95
F8	285.45	67.74
F9	267.74	66.32
F10	**215.47**	**74.66**
F11	240.36	68.88
F12	265.65	66.74

Average of three determinations (n=3)

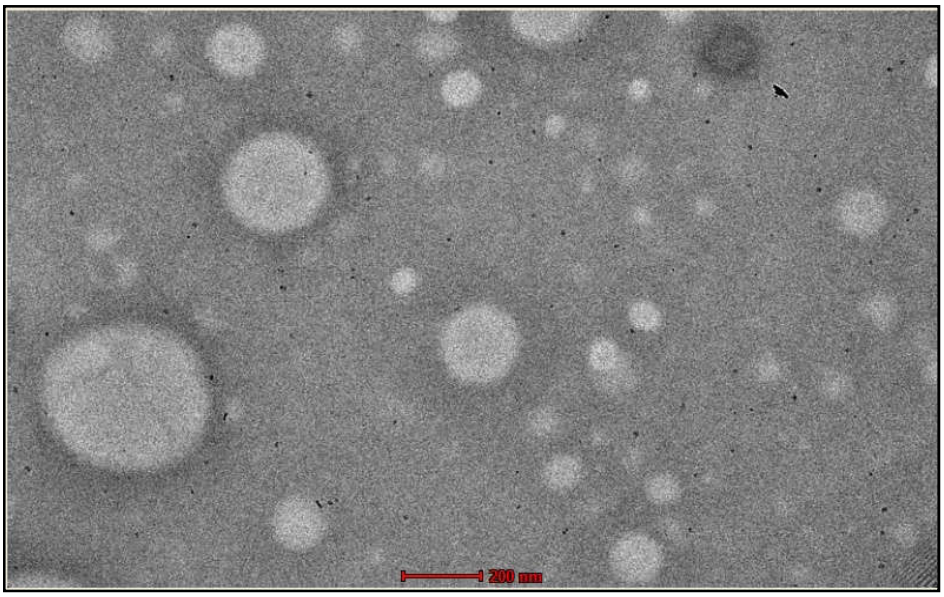

Figure 1: TEM image of phytosomes

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	28.89	1.461	71.11	1.852
1	1	0	38.98	1.591	61.02	1.785
2	1.414	0.301	53.32	1.727	46.68	1.669
4	2	0.602	76.45	1.883	23.55	1.372
6	2.449	0.778	85.56	1.932	14.44	1.160
8	2.828	0.903	93.32	1.970	6.68	0.825
12	3.464	1.079	98.78	1.995	1.22	0.086

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	0.8475	0.9912	0.9544	0.7929

CONCLUSION

In conclusion, the hydroalcoholic extract of *Putranjiva roxburghii* demonstrated significant bioactive content, including flavonoids and phenolics**,** which contributed to its antioxidant potential. The phytosomal formulation of this extract (specifically F10) showed enhanced bioavailability with **a** small particle size and high entrapment efficiency**,** making it a promising candidate for further development as a drug delivery system. The sustained drug release and favorable stability characteristics of the formulation make it a viable option for therapeutic applications**,** particularly in conditions requiring antioxidant or anti-inflammatory effects. Future studies could focus on in vivo evaluations and clinical trials to confirm the efficacy of the phytosomal formulation of *Putranjiva roxburghii* in treating various health conditions.

DECLARATION OF INTEREST

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

REFERENCES

- Agarwal, S. and Dubey, P. (2022) Optimization of phytosomal formulations of flavonoids: An investigation into the use of natural antioxidants. *Pharmaceutical Development and Technology*, 27, 395–404.
- Hung, W.C., Lee, M.T., Chen, F.Y. and Huang, H.W. (2007) The condensing effect of cholesterol in lipid bilayers. *Biophysical Journal*, 92, 3960–3967.
- Kidd, P.M. (2009) Bioavailability and activity of phytosome complexes from botanical polyphenols: The silymarin, curcumin, green tea, and grape seed extracts. *Alternative Medicine Review*, 14, 226–246.
- Kokate, C.K., editor Practical Pharmacognosy (1994), 4th edn, *Vallabh Prakashan*:, 112, 120.
- Mishra, A.G., Singh, R., Patil, N. and Parkhe, G. (2017) Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superb* seed extract. *Asian Journal of Pharmaceutical Education and Research*, 6, 12–17.
- Mukherjee, P.K. (2007). *Quality Control of Herbal Drugs*, 2nd edn, Business Horizons, pp. 2–14.
- Parkhe, G. and Bharti, D. (2019) Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of *Vitex trifolia* Linn. *Journal of Drug Delivery and Therapeutics*, 9, 705–707.
- Patel, S. and Choudhary, P. (2020) Phytochemical analysis and antioxidant activity of Putranjiva roxburghii. *International Journal of Pharmaceutical Sciences and Research*, 11, 1054–1060.
- Reddy, L.H. and Sharma, R. (2015) Phytosomal drug delivery systems: An emerging strategy for the bioavailability enhancement of herbal medicines. *Indian Journal of*

Pharmaceutical Sciences, 77, 163–169.

- Sahu, R. and Verma, A. (2021) Phytosomes: A promising approach for enhancing bioavailability and stability of herbal compounds. *Journal of Drug Delivery Science and Technology*, 61, 102162.
- van Dijk, C., Driessen, A.J. and Recourt, K. (2000) The uncoupling efficiency and affinity of flavonoids for vesicles. *Biochemical Pharmacology*, 60, 1593–1600.
- Yadav, R.S. and Kumar, A. (2019) Putranjiva roxburghii: A review of its phytochemistry, pharmacology, and therapeutic potential. *Journal of Medicinal Plants Research*, 13, 45–55.