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FORMULATION AND EVALUATION OF PHYTOSOMES FOR ENHANCEMENT OF BIOAVAILABILITY OF ETHANOLIC EXTRACT OF *PISTACIA LENTISCUS* L.

Ankita Pal, Diksha Bundela, Pratyush Jain, Nishi Prakash Jain

RKDF College of Pharmacy, SRK University, Bhopal (M.P.)

***Correspondence Info:**
Ankita Pal
RKDF College of Pharmacy,
SRK University, Bhopal (M.P.)
Email: ankitapal4797@gmail.com

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ABSTRACT

This study explores the formulation and evaluation of phytosomes as a novel strategy to enhance the bioavailability of the ethanolic extract of *Pistacia lentiscus* L., a plant renowned for its therapeutic potential. Phytosomes, complex structures formed by combining natural phospholipids with bioactive compounds, offer an innovative solution to address the limited bioavailability of the plant extract. Characterization, *in vitro* release studies, and pharmacokinetic assessments were conducted to evaluate the effectiveness of the phytosomal formulation. The results indicate a significant improvement in bioavailability, suggesting the clinical relevance of this approach. Safety and tolerability assessments further support the potential utility of phytosomes for enhancing the therapeutic applications of *Pistacia lentiscus* L. extract.

Key words: *Pistacia lentiscus*, Phytosomes, Formulation, Characterization

INTRODUCTION

Pistacia lentiscus L., commonly known as mastic, has a long history of use in traditional medicine due to its various pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial effects (Milia *et al.,* 2021). However, the clinical utility of *Pistacia lentiscus* L. extract has been hindered by its poor bioavailability, primarily attributed to its low water solubility and limited absorption. Efforts to enhance the bioavailability of this valuable botanical extract have prompted the exploration of innovative delivery systems (Gacem *et al.,* 2020; Pachi *et al.,* 2020).

Phytosomes have emerged as a promising approach to improve the solubility, stability, and bioavailability of poorly water-soluble bioactive compounds. These specialized complexes, formed by combining phospholipids with bioactive molecules, offer a means to overcome the limitations associated with traditional formulations (Bhattacharya, 2009). By encapsulating the ethanolic extract of *Pistacia lentiscus* L. within phytosomes, it is possible to enhance its dissolution rate, protect it from degradation, and facilitate its absorption in the gastrointestinal tract (Saroha, 2021).

In this context, the present study aims to formulate and evaluate phytosomes as a delivery system for *Pistacia lentiscus* L. extract, with the primary goal of enhancing its bioavailability. The research involves the preparation of phytosomes, their detailed characterization using various analytical techniques, in vitro release studies to assess the release kinetics, and pharmacokinetic assessments to determine the impact on bioavailability. Safety and tolerability evaluations are also conducted to ensure the

suitability of the phytosomal formulation for therapeutic applications.

By investigating the potential of phytosomes to improve the bioavailability of *Pistacia lentiscus* L. extract, this study contributes to expanding the clinical utility of this natural remedy. The results obtained herein may have implications for the development of more effective and accessible treatments harnessing the therapeutic potential of mastic (Khanzode *et al.,* 2020).

MATERIALS & METHODS

Extraction of plant materials by maceration method

The defatted plant drugs were subjected to extraction by ethanol solvent. The liquid extracts were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated (Mukherjee, 2007; Khandelwal, 2005).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. The % yield was calculated by using formula:

% yield = [(weight of dried extract) / (weight of dried plant sample)] x 100

Phytochemical screening

Test for alkaloids

1. Hager's test: to a few ml of filtrate, 2 drops picric acid was added formation of yellow precipitate shows a positive result for alkaloids.

2. Wagner's test (iodine – potassium iodine reagent): To about an ml of extract few drops of Wagner's reagent were added. Reddish – brown precipitate indicates presence of alkaloids (Kokate, 1994).

2. Test for phenol

A) FC reagent test: To 5ml of extract 2ml of Folin–Ciocalteu reagent is added. Appearance of blue green colour indicates the presence of phenol.

B) Ferric chloride test: To 5 ml of extract few drops of feeric chloride solution was added and mixed gently. The production of blueish black colour solution indicate presence of phenols.

3. Test for flavonoids

A) Alkaline reagent test: To 5ml of extract 2ml of NaOH was added by which solution turns yellow colour, further dilute HCl (0.1N) was added the solution becomes colourless which indicates the presence of phenol.

B) Lead acetate test: To 5 ml of extract few drops of lead acetate solution was added and mixed gently. The production of bulky white precipitate is positive for flavonoid.

4. Test for carbohydrate

A) Benedict's test: About 0.5 ml of the filtrate was taken to which 0.5 ml of Benedict's reagent is added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

B) Fehling test test: About 0.5 ml of the filtrate was taken to which 0.5 ml of each Fehling A & Fehlinng B solution was added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

5. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

6. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of glycosides: Extract was treated with dil. H_2SO_4, formation of red color solution indicate the presence of glycosides.

7. Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

8. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Quantitative estimation of phenols and flavonoids

Estimation of total phenolic content

Folin-Ciocalteu (FC) colorimetric method is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. In the present investigation, Folin-Ciocalteu (FC) colorimetric method is employed for the quantitative estimation of total phenolic content present in ethanolic extract of *Pistacia lentiscus* (Mishra *et al.,* 2017).

Procedure

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Procedure

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared (Mishra *et al.,* 2017). 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Formulation development of phytosomes

The complex was prepared using a mixture of phospholipids, cholesterol, and *Pistacia lentiscus* extract in different ratios: 1:0.5:1, 1:1:1, 2:1.5:1, and 2:2:1, as described by Kidd in 2009. The following steps were followed:

- Weighted amounts of the extract, phospholipids, and cholesterol were placed in a 100ml round-bottom flask.
- 25ml of dichloromethane was added to the flask as the reaction medium.
- The mixture was refluxed, and the reaction temperature was maintained at 50°C for 3 hours.
- After the reaction, the resulting mixture became clear.
- The clear mixture was then evaporated, and 20 ml of n-hexane was added to it while stirring.
- The precipitate that formed was filtered, and any remaining traces of solvents were removed by drying under vacuum.
- The dried residues were collected and placed in a desiccator overnight to remove any residual moisture.
- Finally, the prepared complexes were stored at room temperature in amber-colored glass bottles. These steps were taken to synthesize the complexes with the specified ratios of components for further analysis or use.

Table 1: Different formulations of phytosomes

F. code	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 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 *Pistacia lentiscus* 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 *Pistacia lentiscus* extract in 1 ml dispersion. The percent entrapment was calculated by following formula.

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

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.

***In vitro* dissolution rate studies**

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type) (Higuchi, 1963). 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.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of *Pistacia lentiscus* ethanolic extract reveals the presence of several primary and secondary metabolites, including carbohydrates, amino acids, proteins, diterpenes, glycosides, saponins, flavonoids, and tannins/phenols. These compounds may contribute to the potential medicinal properties and biological activities of the extract. However, the absence of steroids, triterpenoids, volatile oils, and alkaloids suggests that these specific classes of compounds are not prominent in this extract. Further studies and detailed chemical analysis are necessary to determine the exact composition and potential therapeutic uses of the extract table 2.

Table 3 presents the total bioactive constituents content of *Pistacia lentiscus* in terms of total phenol and total flavonoid content, measured in milligrams per 100 milligrams of the extract.

Phenols are a group of chemical compounds that are known for their antioxidant properties. They play a crucial role in protecting cells from oxidative stress and can have various health benefits. In this case, the ethanolic extract of *Pistacia lentiscus* contains 0.758 mg of phenols per 100 mg of the extract. This indicates a moderate level of phenolic compounds in the extract, suggesting potential antioxidant activity. Ethanolic Extract - total flavonoid content (0.961 mg/100mg): Flavonoids are a subclass of phenolic compounds known for their antioxidant, anti-inflammatory, and other health-promoting properties. The ethanolic extract of *Pistacia lentiscus* contains 0.961 mg of flavonoids per 100 mg of the extract. This is a relatively higher amount of flavonoids compared to the phenol content, suggesting that flavonoids may be a significant contributor to the antioxidant potential of the extract.

The data provided in table 4 suggest that several of the formulations (particularly F2, F6, and F10) exhibit desirable characteristics in terms of particle size and entrapment efficiency for drug delivery applications.

These formulations may offer improved drug bioavailability and efficient drug loading within the phytosomes. The choice of the optimal formulation will depend on various factors, including the specific drug being delivered, its intended therapeutic effect, and the desired release profile. Further studies and in vitro/in vivo testing would be necessary to assess the performance of these formulations in real-world applications. The high R^2 values for the First Order and Higuchi models suggest that these models provide strong fits to the drug release data for formulation F10. This suggests that drug release from F10 may predominantly follow first-order kinetics and may also involve a diffusion-based mechanism. The Korsmeyer-Peppas model also provides a reasonably good fit, indicating that the release behavior may be more complex and involve both diffusion and potentially other mechanisms table 6.

Table 2: Preliminary qualitative phytochemical tests for *Pistacia lentiscus* extract

Phytoconstituents	*Pistacia lentiscus* ethanolic extract
i)Primary Metabolites	
Carbohydrates	(+)
Amino acids	(+)
Proteins	(+)
Fats and oils	(-)
ii)Secondary metabolites	
Steroids	(-)
Triterpenoids	(-)
Volatile oils	(-)
Diterpenes	(+)
Glycosides	(+)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(-)
EE = Ethanolic extract; '+' = Present; '-' = Absent	

Table 3: Total bioactive constituents content of *Pistacia lentiscus*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Ethanolic extract	0.758	0.961

Table 4: Particle size and entrapment efficiency of drug loaded phytosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	345.65	63.32
F2	**263.32**	**73.32**
F3	315.45	69.92
F4	340.11	68.74
F5	285.45	65.52
F6	**252.23**	**73.15**
F7	278.85	70.22
F8	293.32	68.98
F9	269.95	63.32
F10	**220.23**	**72.25**
F11	236.65	68.85
F12	248.85	66.65

Average of three determinations (n=3)

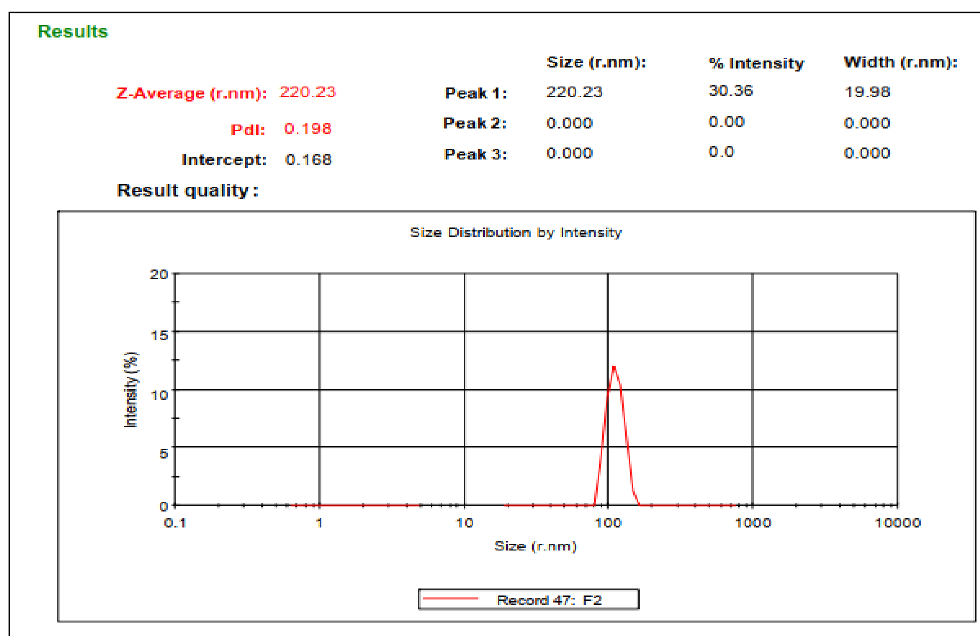

Figure 1: Particle size of optimized batch F10

Table 5: *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	25.65	1.409	74.35	1.871
1	1	0	36.65	1.564	63.35	1.802
2	1.414	0.301	48.85	1.689	51.15	1.709
4	2	0.602	72.23	1.859	27.77	1.444
6	2.449	0.778	89.98	1.954	10.02	1.001
8	2.828	0.903	96.65	1.985	3.35	0.525
12	3.464	1.079	99.45	1.998	0.55	-0.260

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	0.847	0.988	0.949	0.899

CONCLUSION

In conclusion, the formulation and evaluation of phytosomes as a delivery system for the ethanolic extract of *Pistacia lentiscus* L. have shown promising results for enhancing bioavailability. This innovative approach addresses the limitations associated with the poor solubility and absorption of the extract. Overall, the development of phytosomes for enhancing the bioavailability of the ethanolic extract of *Pistacia lentiscus* L. holds promise for expanding the clinical applications of this natural remedy. Further research may be needed to explore the full therapeutic potential and optimize the formulation for specific medical conditions.

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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