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RESEARCH ARTICLE

Formulation Development and *In Vitro* Characterization of *Gymnema sylvestre*'s Phytochemical Encapsulated Phytosome

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Abstract

Background: Drug therapy for Type II diabetes mellitus suffers from a frequent administration of oral anti-diabetic drugs, which leads to drug tolerance and nephrotoxicity as major side effects. **Aim:** To address this issue, the current research focused on investigating gymnemic acid and its subsequent encapsulation in phytosomes on the release properties of anti-diabetic drugs. In this research work, gymnemic acid shall be encapsulated in a phospholipid bilayer, it is called a Phytosome and it will be thoroughly characterized through *in vitro* study. **Methods:** Phytosomes with gynemic acid encapsulation have to be created using the solvent evaporation method. In order to preserve their stability, the created phytosomes were lyophilized. Then the gymnemic acid encapsulated phytosome was characterized for Measurement of Zeta potential, Measurement of particle size, Determination of entrapment efficiency, Scanning electron microscopy, Drug content and *In-vitro* Drug release study. **Results:** The drug content and entrapment efficiency of the best formulation (F3) was found to be 43.41% and 78.43%. **Conclusion:** The study's findings indicate that gymnemic acid may release the medication for a longer period and significantly lower blood glucose levels after being encapsulated into phytosomes.

Keywords: Anti-diabetic Activity, *Gymnema sylvestre* Leaves, Phytosomes

1. Introduction

The plant is indigenous to tropical Africa, Australia, and central and western India[1]. The utilization of all plant parts is essential in the treatment and cure of various ailments, while also playing a crucial role in the development of numerous pharmaceutical formulations. The plant has a long history of usage in naturopathic diabetic treatment[2,3]; it also shows promise in the treatment of arthritis, hyperlipidemia, obesity, high cholesterol and Parkinson's illness[4,5,6]. The bioactive substances found in plants also have antibacterial, anti-inflammatory and anticancer activities. The plant's leaves are used to cure rheumatism,

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obesity[7], dental cavities[8], stomach aches and blood purification and rheumatism[9]. Figure 1 exhibits the leaves of the *Gymnema sylvestre* plant.

Gymnemic acid's ability to mimic the atomic configurations of sugar molecules and block taste bud receptors makes it a promising natural ingredient for reducing sugar cravings and promoting healthier eating habits. Similarly, it attaches itself to a receptor in the intestine's outer layer, preventing the colon from receiving molecules of sugar and reducing blood sugar levels[10].

2. Materials and Methods

2.1 Materials

Gymnema sylvestre botanical matter was gathered from Sankaralingam Bhuvaneshwari College of Pharmacy, herbal garden, Sivakasi, Tamil Nadu. It is authenticated from Ayya Nadar Janaki Ammal College, Botany Department, Sivakasi, Tamil Nadu. *Soya lecithin* was procured from Molchem Chemical Laboratories, Mumbai, India. Cholesterol was procured from Nice Chemicals (P) Ltd., Kochi, India. Ether was procured from Avantor Performance Materials India Limited. Methanol was procured from Reachem Laboratory Chemicals Private Limited, Chennai, India.

2.2 Methods

Gymnema sylvestre leaves are cleaned. The dried leaves were powdered and then passed through 40 mesh and the organoleptic properties of colour, and odour taste were evaluated. The solubility of *G. sylvestre* dried leaves powder was determined by using water, ethanol, methanol, chloroform and pH 6.8 buffer[11].



Figure 1. Leaves of *Gymnema sylvestre.*

2.2.1 Extraction

100 g of dry plant leaves shall be pulverized and extracted with 1000 ml of 90% methanol by using a soxhlation extraction process. The entire methanol solvent extract must be achieved after 24 h of extraction. The extract shall be subjected to evaporation using a heating mantle. The thick paste of phytoconstituent shall be collected after evaporating process[12].

2.2.2 Isolation

On constant stirring for 30 to 50 min, a 20 g thick compound of methanol easily soluble extract was dispersed in one per cent aqueous potassium hydroxide solution. The undissolved particles are subsequently separated from the solution by filtering it through filter paper. Gymnemic acids precipitated while diluted HCl was gradually added with steady stirring. To get purified gymnemic acid, this collected liquid was passed through a filter and dried using pressure[13].

2.2.3 Phytochemical Screening Study

The phytochemical screening study of phenols, glycosides, and steroids was determined[14]. The isolated gymnemic acid was identified by the Thin Layer Chromatographic method[15].

2.2.4 FT-IR Spectroscopy

This technique may be utilized to investigate and predict possible physical interactions between excipients in a formulation and gymnemic acid[16,17].

2.3 Standard Calibration Curve of Gymnemic Acid in UV Spectrophotometer

Gymnemic acid standard solution within a buffer with phosphate pH 6.8 demonstrated linearity for Ultraviolet (UV) radiation within a spectrum of 10 to 60 g/ml of medication for an optimal range of 426nm. Absorbance was plotted on the y-axis against concentration on the x-axis to get the typical graph shown in Figure 2. The slope was found to be with the R2 value of 0.999.

3. Formulation of Gymnemic Acid Encapsulated Phytosome

The dispersion method was selected for the phytosome formulation.

3.1 Dispersion Method

The procedure involved the dissolution of soy lecithin and cholesterol in ether. It acts as an oil phase. Then the extracted plant medicaments are dissolved in phosphate buffer pH 6.8. The aqueous medium is placed under a magnetic stirrer for 5-10 min. After the complete dissolution of medicaments, the oil phase was added into an aqueous medium dropwise. Then the magnetic stirrer is allowed to stir for 1 h with 1000rpm. Then the product will be collected. The prepared phytosomal suspension should be processed around 8,000 revolutions per minute for 40 min at 40 °C. After being diluted with distilled water, a precipitate was used in evaluation studies[18]. The formulation of Gymnemic acid phytosome by dispersion method was presented in Table 1.

3.2 Lyophilization

Moisture gets removed from a frozen product before it is placed in a vacuum, permitting the product to transition right away from solid to vapour without passing through the phase of liquid. This is referred to as lyophilization or freeze drying[19].

3.3 Characterization of Gymnemic Acid Encapsulated Phytosomes

3.3.1 Scanning Electron Microscopy (SEM)[20]

Gymnemic acid phytosome formulations' surface appearance, size, and shape were studied using scanning electron microscopy. The improved freeze-dried phytosomes were photographed and subjected to scanning electron microscopy.

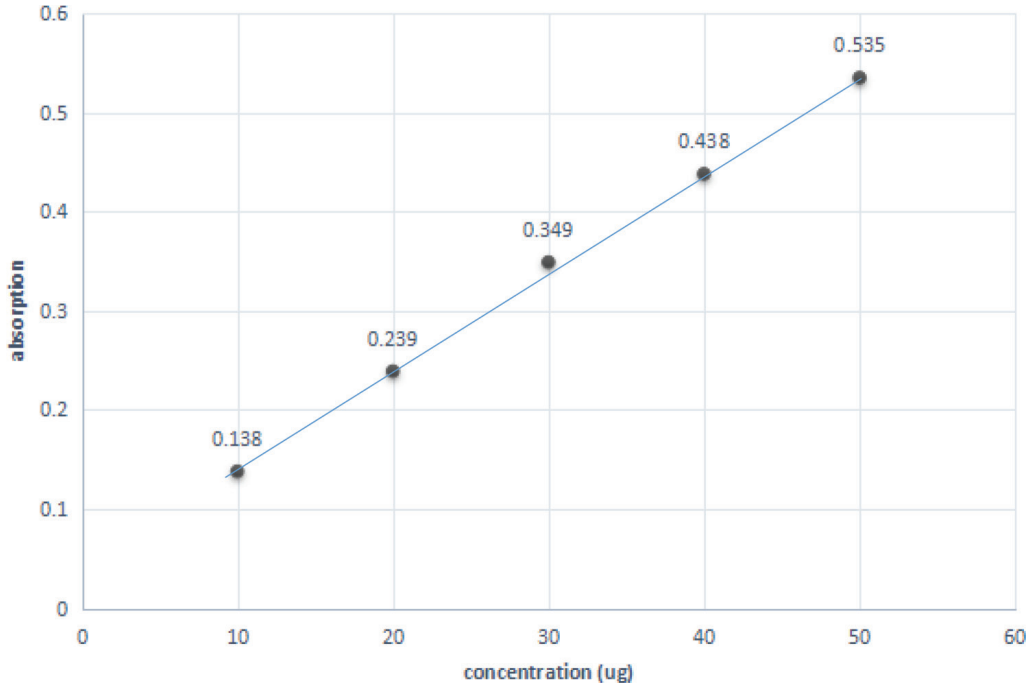


Figure 2. Standard calibration curve of gymnemic acid.

Table 1. Formulation of gymnemic acid phytosome by dispersion method

Formulation Code	Drug (mg)	Cholesterol (mg)	Soya Lecithin (mg)	Ether (mL)	Phosphate Buffer pH 6.8 (mL)
F1	500	500	500	10	10
F2	500	1000	1000	10	10
F3	500	1500	1500	10	10
F4	500	2000	2000	10	10
F5	500	2500	2500	10	10
F6	500	3000	3000	10	10

3.3.2 Measurement of Particle Size (PS)[21]

Gymnemic acid phytosomes' particle size was identified using a particle size analyzer. A suitable volume of PBS pH 6.8 was used to dilute 300µm of the formulation for the measurement, and the vesicle diameter was then calculated.

3.3.3 Measurement of Zeta Potential (ZP)[22]

The most crucial factor affecting the physical stability of phytosomes is Zeta potential. This Zeta potential value of 20 mV predicts favourable physical stability for the distribution. The Microtrac was used to test the Zeta potential of the enhanced phytosome suspension. To measure the Zeta potential, just one millilitre from the material being studied was diluted with water to create 10 ml, and then 5 ml from the sample was diluted and transferred to a cuvette.

3.3.4 Determination of Drug Content[23]

The amount of medication in the complex was determined by accurately weighing 10 mg of the phytosome complex and dissolving it in ten millilitres of methanol. After the proper dilution, the drug content was ascertained by measuring the absorbance using a UV spectrophotometer at 426 nm.

3.3.5 Entrapment Efficiency (EE)[24]

Gymnemic acid phytosomes were rotated at twelve thousand revolutions per minute for 45 min with a Remi centrifugation to separate them from unentrapped medicines. The concentration of free medicine in the supernatant was determined by measuring fluorescence around 426 wavelengths in an ultraviolet (UV)--visible spectrophotometer. Using the formula, the percentage of drug entrapment was determined.

$$Entrapment\ Efficiency(\%) = \frac{Amount\ of\ encapsulated\ drug}{Amount\ of\ drug\ added} \times 100$$

3.3.6 In Vitro Drug Release Studies[25]

The drug release was determined using a prepared cellophane membrane put on the end of a hollow tube containing the phytosome (equivalent to 50 mg of gymnemic acid). The phosphate buffer was 6.8 and 250 ml of a 500 ml beaker in which the dialysis tube was suspended. The solution was stirred at one hundred revolutions per minute using a magnetic stirrer around 37 °C. After specified times, a single millilitre of the collected material was discarded and replaced with equal amounts of new PBS. Each sample was filtered, diluted, and put through a UV spectrophotometer for evaluation.

4. Results and Discussions

The study's purpose was to develop gymnemic acid-encapsulated phytosomes that would deliver medication for an extended period under controlled conditions. This phytosomes were created using a dispersion method. The different ratios of 1:1:1, 1:2:2, 1:3:3, 1:4:4, 1:5:5 and 1:6:6 were used for encapsulating the drug. Diethyl As a solvent, ether was employed. As a hydrating media for loading the medication, phosphate buffer pH 6.8 was utilized.

4.1 Preformulation Studies

4.1.1 Organoleptic Properties

The dried leaf powder o*f G. sylvestre* looks green in colour; it has no specific odour and is bitter in taste.

4.1.2 Solubility

Gymnema sylvestre dried leaf powder was discovered that it was insoluble in water, Methanol, chloroform and buffer pH 6.8 are all easily soluble, but ethanol is only sparingly soluble.

4.2 Extraction

In the soxhlation extraction process, a yield of 18 g of phytoconstituents was obtained**.**

4.3 Isolation

Since the leaves of *G. sylvestre* contain several phytoconstituents, only gymnemic acid was extracted using a precipitation process. A yield of 8 g of pure gymnemic acid was obtained.

4.3.1 Chemical Test

Gymnemic acid passed the phenolic, steroid, and glycoside tests. A rich blue colour occurred through the phenolic test, indicating that of a -OH group in the molecule. In the steroids test, the top level turns red, while the sulphuric acid layer goes yellow with green

fluorescence. At the confluence of the two layers, a bluish-red ring formed, showing the presence of glycoside.

4.3.2 Thin Layer Chromatography

To support phytochemical screening, gymnemic acid was subjected to a thin layer of chromatography. The chromatography results revealed that gymnemic acid exhibited excellent separation and retention properties within the Isopropyl alcohol: Methanol: Chloroform: Acetic acid (5:1:3:0.5) mobile phase, which can be valuable in pharmaceutical and analytical applications. TLC findings were comparable with the data of qualitative chemical tests and the spots characteristic of gymnemic acid. TLC analysis found that the characteristics are identical to the typical gymnemic acid with Rf 0.76.

4.4 Compatibility Studies

The FTIR peak matching method was used to assess the compatibility of gymnemic acid, Soya lecithin, and cholesterol. Peaks did not arise or disappear in this drug-lipid combination, demonstrating the lack of a chemical connection between the medication as well as the fats as demonstrated in Figure 3.

It is observed here that no appreciable interaction exists between API and other ingredients in the formulation therefore the formulation does not have any interaction between other ingredients and API. Hence, FT-IR studies further supported the absence of incompatibility between drugs and excipients.

4.5 Preparation of Gymnemic Acid Phytosomes Complex

Gymnemic acid phytosomes were prepared by dispersion method in the ratio of 1:1:1, 1:2:2, 1:3:3, 1:4:4, 1:5:5 and 1:6:6 by varying the concentration (Figure 4).

4.6 Lyophilization

The formulated phytosomes were lyophilized to maintain their stability. After the lyophilization process, the yield was 5 g of Gymnemic acid in the phytosome.

4.7 Characterization of a Phytosomes

4.7.1 Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM) analysis of these gymnemic acid phytosomes revealed the presence of sphere-shaped vesicles as shown in Figure 5.

4.7.2 Drug Content and Entrapment Efficiency

The percentage yield of gymnemic acid phytosomes F3 contains gymnemic acid, When examined alongside F1, F2, F4, F5, and F6, cholesterol and lecithin from soya under the proportion of 1:3:3 had the highest yields in percentages. The drug content of the phytosome complexes was determined. The percentage of drug content ranged between 16.38 and 43.41%. The phytosome complexes' entrapment efficiency was investigated and presented in Table 2. The percentage efficiency of entrapment is around

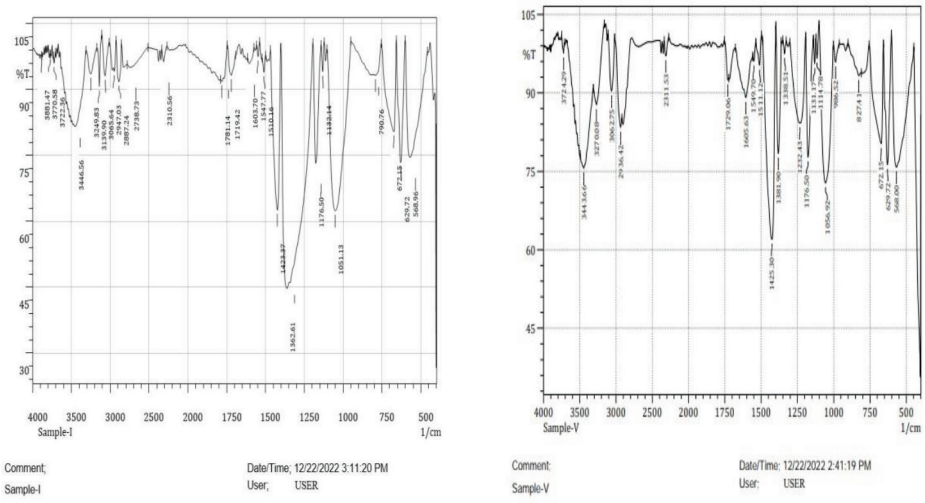


Figure 3. FT-IR spectra of *G. sylvestre* extract, soya lecithin and cholesterol.

78.43%. Studies demonstrated increased drug quantity and entrapment efficiency.

4.7.3 In-Vitro Drug Release

These phytosomes were placed in a diffusion cell, and then the space for receptors was filled using a pH 6.8 solution of buffer. The diffusion tube had been kept around 37±5 degrees Celsius. The phytosome corresponding to 50 mg of gymnemic acid was used in all of the produced formulations. F3 cumulative medication release was reported to be 96.23%. *In-vitro* gymnemic acid release showed a very rapid first burst, preceding a highly delayed drug delivery (Figure 6). The study starts, with rapid release suggesting that a certain medication has been concentrated on the surface of the phytosomes and presented in Table 3.

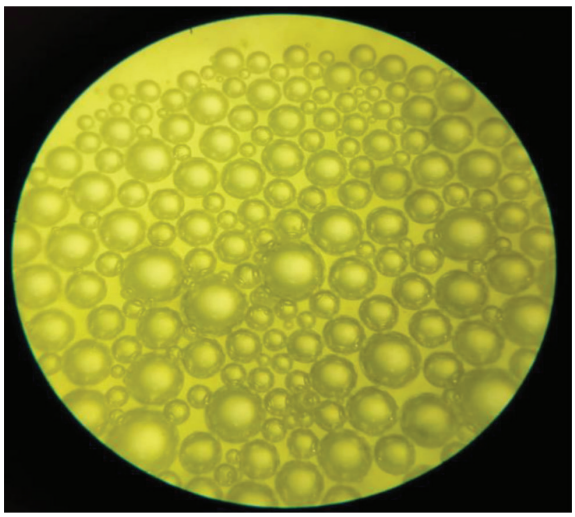


Figure 4. Microscopic view of gymnemic acid encapsulated phytosome by dispersion method.

Table 2. Formulation code with percentage drug content and percentage entrapment efficiency of gymnemic acid phytosomes

S. No.	Formulation Code	Percentage Drug Content (%w/w)	Percentage Entrapent Efficiency
1	F1	16.38	65.06
2	F2	31.49	71.67
3	F3	43.41	78.43
4	F4	39.48	75.15
5	F5	32.31	71.86
6	F6	34.17	72.63

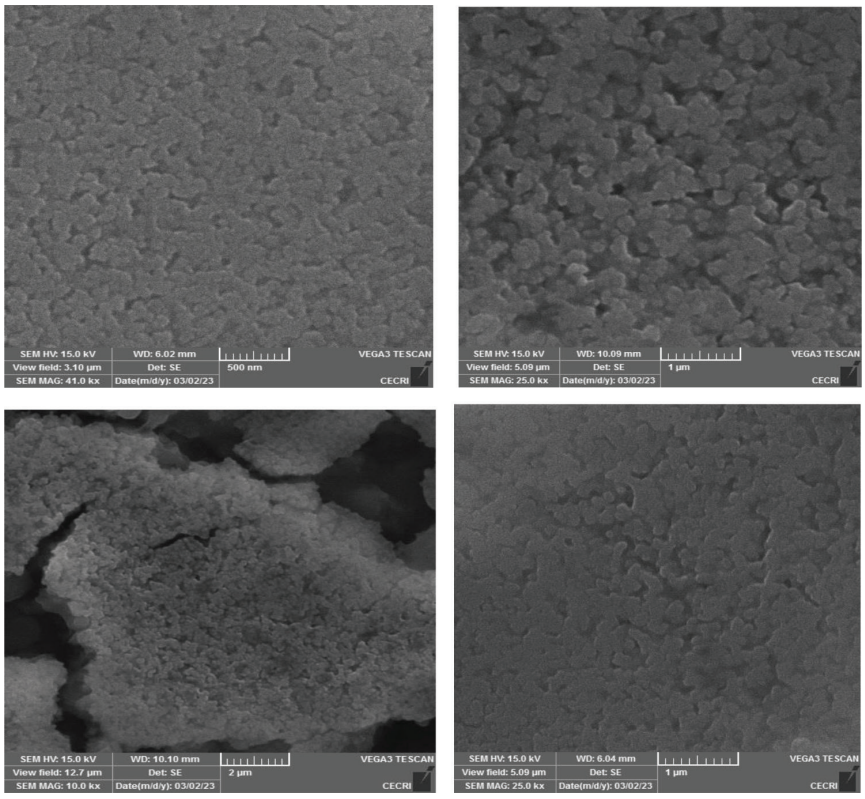


Figure 5. SEM of Gymnemic acid Phytosomes F3.

Table 3. Cumulative percentage drug released of gymnemic acid encapsulated phytosomes

S. No.	Time (mins)	Pure Drug	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
1	10	25.61	22.37	26.42	24.76	15.81	18.34	20.84
2	20	35.04	37.29	38.47	36.29	31.47	33.84	35.73
3	30	50.63	49.36	50.29	48.35	42.83	45.82	47.26
4	40	80.34	76.21	78.14	76.93	75.02	77.11	79.84
5	50	99.02	94.03	98.56	96.65	91.67	92.54	93.71

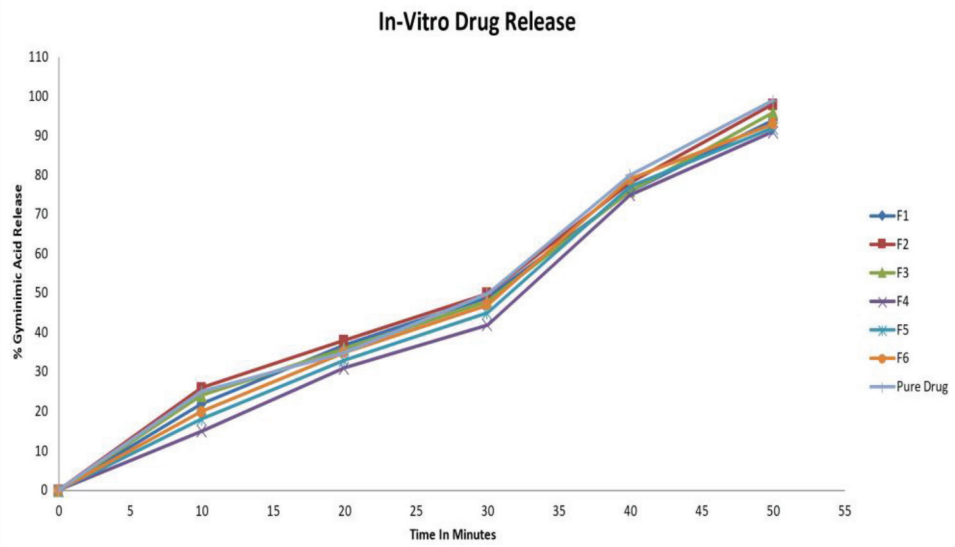


Figure 6. *In vitro* drug release of gymnemic acid phytosomes F1-F6.

5. Discussion

The amount of medication released in 48 h for all formulations F1, F2, F3, F4, F5 and F6 was 94.03%, 98.56%, 96.65%, 91.67%, 92.54%, and 93.71%, respectively. The formulation F2 showed shorter release than formulation F1, F3, F4, F5 and F6. The produced phytosomes of F1, F3, F4, F5 and F6 demonstrated Sustained drug release.

6. Conclusion

This study concluded that the plant leaves of *G. sylvestre* were extracted using methanol. It had many phytoconstituents like phenols, steroids and glycoside. The extraction of *G. sylvestre* was isolated to separate, gymnemic acid by Precipitation method. The gymnemic acid phytoconstituent was identified by the TLC method. FT-IR studies further supported the absence of incompatibility between drugs and excipients. By using a dispersion process, pure gymnemic acid was successfully synthesized as a phytosome drug delivery system. Cholesterol and soya lecithin were administered in varied concentrations (1:1:1, 1:2:2, 1:3:3, 1:4:4, 1:5:5 and 1:6:6). The formulated phytosomes were lyophilized to maintain their stability. Thus the gymnemic acid-encapsulated phytosomes were formulated and evaluated for various parameters. SEM results showed formulation (F3) was spherical. The presence of hydrogen-bonded or the forces of van der Waals in the complexation with gymnemic acid and phospholipids has been proven by the size of particles and Zeta potential. F3 was chosen as the best formulation based on drug content and entrapment efficiency. In this study, we observed that the gymnemic acid-encapsulated phytosomes released the drug for a long period. Compared to others, F4 has a longer 48 h drug release duration.

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8. Author contribution

E Bhavya: Visualization, Supervision; P Inigo: Conceptualization, Writing - Original Draft; M S Arun Kumar: Methodology, Formal analysis, Writing - Original Draft; P Surya Vijay: Investigation; R Anandaraj: Validation; A Rajalakshmi: Data Curation; M Sona: Writing - Review and Editing.

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