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Original Article

Formulation and Development of *Euphorbia hirta* Phytosomes Capsule for Treatment of Urinary Tract Infection

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ABSTRACT

Background: *Euphorbia hirta*, also known as the "asthma plant" or "snake weed," belongs to the *Euphorbiaceae* family. However, its oral use as a drug has certain limitations, such as low bioavailability and high first-pass metabolism. Traditional treatment options like tablets and capsules available in the market often require high doses and frequent administration. To overcome these limitations, researchers aimed to develop an improved drug delivery system using *Euphorbia hirta* in the form of phytosomes. **Materials and Methods:** Phytosomes of *Euphorbia hirta* Extract (EHE) were developed using Thin Film Hydration Technique and evaluated them through different tests, such as Scanning Electron Microscopy (SEM), drug entrapment, in addition to *in vitro* release investigations. **Results and Conclusion:** Amongst the formulations tested, F9 exhibited the best drug content and entrapment efficiency, with a drug entrapment of 95.89%. The *in vitro* release studies showed that the formulated phytosomes, particularly formulation F9, achieved the desired drug release of 49.60% over an extended period of 12 hr. Subsequently, the selected formulation was encapsulated into capsules and compared with the available market product, demonstrating sustained release properties with 63.20% drug release over 12 hr. In conclusion, formulation F9 successfully transformed the crude extract of *Euphorbia hirta* into a novel targeted drug delivery system in the form of phytosomes. This system holds promise for effectively treating medically challenging diseases like Urinary Tract Infections (UTI).

Keywords: Urinary Tract Infection, *Euphorbia hirta*, Phytosomes, Capsule.

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INTRODUCTION

Throughout the course of human civilization, individuals have depended on natural substances, particularly botanicals, to promote well-being, address ailments, and alleviate discomfort. Recently, there has been a growing awareness of traditional knowledge about medicinal plants, leading to a shift from allopathic medicines to herbal remedies. This trend is evident in the increasing number of physicians and public health specialists worldwide who are exploring alternative plant-based treatments (Hoareau *et al*., 1999; Cordell *et al*., 2000). Currently, there is a worldwide endeavor to discover ecologically sound, non-harmful, and economically feasible plant-derived solutions for treating diverse medical conditions. The rising popularity of herbal remedies can be attributed to multiple factors (Arora *et al*., 2005; Said *et al*., 2002; Farnsworth *et al*., 1995).

Traditional medicines and phyto-medicines have been widely prevalent since ancient times for maintaining health through various means (Abhinav *et al*., 2016; Patel *et al*., 2010). In current times, there have been noteworthy developments in herbal drug delivery aimed at efficiently managing human diseases (Gold *et al*., 2000). Across the globe, nations are seeking healthcare solutions beyond the conventional boundaries of modern medicine, leading to an increasing trend of self-medication with herbal remedies (Gold *et al*., 2000).

Medicinal plants continue towards playing a crucial role in improving the quality of life for a large portion of the world's population and are an essential portion of the healthcare system

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(Soejarto, 1996). However, formulating plant extracts into dosage forms is a complex process that goes beyond mere pharmaceutical technology (Kamboj, 2000). Researchers are actively working on formulating plant extract-based products and improving existing ones to address issues like poor absorption, rapid metabolism, and rapid systemic elimination. This requires the implementation of numerous approaches to overcome these challenges (Soejarto, 1996; Goswami and Fernandes, 2002).

Most phyto-medicines contain bioactive constituents that are water-soluble molecules, such as phenolics, glycosides, and flavonoids. Nevertheless, the effectiveness of these water-soluble phytoconstituents is limited due to poor absorption when taken orally or applied topically (Yang *et al.*, 2008). This poor absorption is mainly attributed to their large molecular size, making passive diffusion difficult, or their lower lipid solubility. In contrast, phytosomes, unlike liposomes, have the unique ability to cross lipid-rich bio membranes more effectively (Yang *et al.*, 2008).

Several methods have been devised to improve the absorption of these plant-derived compounds when taken orally. These methods include incorporating solubility and bioavailability enhancers, making structural modifications, and entrapping them with lipophilic carriers. In-depth investigation within the realm of herbal drug delivery systems is essential to enhance the therapeutic efficacy of these drugs in comparison to standard herbal extracts (Amit *et al.*, 2007).

Phytosomes and liposomes are structurally distinct. Phytosomes are small units formed by bonding a few molecules together, while liposomes are aggregates of numerous phospholipid molecules that enclose other active compounds without any specific bonding (Amit *et al.*, 2007). The breakthrough model of phytosomes technology significantly enhances the bioavailability and clinical benefits of phyto-medicines. It ensures the assured delivery of active compounds to tissues while maintaining the safety of nutrients (Kumari *et al.*, 2011; More *et al.*, 2012).

Euphorbia hirta, also known as the "asthma plant," "snake weed," or "garden spurge," is a small herbaceous plant belonging to the *Euphorbiaceae* family. It is indigenous to numerous tropical and subtropical regions worldwide and is often considered a weed due to its fast and widespread growth (Kumar *et al.*, 2010). The plant usually reaches a height of 30-40 centimeters (12-16 inches) and features small green leaves with serrated edges. It produces inconspicuous flowers and small fruit capsules containing seeds (Kumar *et al.*, 2010).

Euphorbia hirta has a long history of use in traditional medicine for various purposes. In some cultures, it has been utilized to address respiratory conditions like asthma, which earned it the name "asthma plant." Additionally, it is believed to possess antifungal, antibacterial, and anti-inflammatory properties. The present study's aim is to formulate and develop a phytosomes capsule using *Euphorbia hirta* for the treatment of urinary tract infections.

MATERIALS AND METHODS

Materials

Euphorbia hirta Extract was received as a generous gift sample from Amsar Goa Pvt. Ltd., India. Soya lecithin was obtained from VAV Lipid Pvt. Ltd., Mumbai, India. Dichloromethane and n-Hexane was acquired from Ostwald Scientific Pvt. Ltd., Pune. Chloroform and Methanol was attained from Molychem, Mumbai. Supplementary chemicals and reagents used were of analytical grade.

Methods

Physico-chemical investigation of Euphorbia hirta Extract

The characteristics of the *Euphorbia hirta* Extract were assessed through a physicochemical investigation, which included evaluating its appearance, color, taste, and odor.

Preliminary phytochemical screening of the extract

The *Euphorbia hirta* Extract was analyzed through a preliminary phytochemical examination to detect the presence of different phytoconstituents, employing established methods as documented in the literature (Sahu and Bothara, 2015; Sharma and Sahu, 2016).

Preparation of Phytosomes of *Euphorbia hirta* Extract with Thin Film Hydration Technique

The phytosomal formulation of *Euphorbia hirta* was prepared using the Thin Film Hydration Technique in conjunction with the Anti-solvent Precipitation method. Initially, a suitable amount of Soy-lecithin and Extract were combined in a round bottom flask and dissolved by adding 20 mL of dichloromethane. The mixture was then refluxed at a controlled temperature below 60°C for 2 hr, causing the organic phase to evaporate and form a thin layer on the flask's inner surface. After this time, the remaining mixture was concentrated to a volume of 5-10 mL. Subsequently, 20 mL of n-Hexane was added to the concentrated solution while stirring continuously, resulting in the formation of a precipitate. The precipitate was filtered, collected, and subjected to overnight storage in a vacuum desiccator to eliminate any remaining solvent traces. The dried precipitate was further crushed and sifted through a #100 mesh sieve to obtain a powdered complex. Finally, the powdered phytosomal formulation was carefully transferred into an amber-colored glass bottle and stored at room temperature for future utilization (Sahu and Bothara, 2015; Sharma and Sahu, 2016).

Formulation Optimization

In the conventional approach to developing pharmaceutical formulations, changes are made to one variable at a time, leading

to a time-consuming and imaginative process. However, this method may not be ideal as it does not consider the shared possessions of independent variables. Consequently, creating an optimal formulation becomes challenging. To address this, it is crucial to employ established statistical tools like factorial designs to comprehend the complexity of pharmaceutical formulations better. Through utilizing factorial designs, researchers can analyze multiple variables simultaneously and gain a deeper understanding of their joint impacts on the formulation, making the development process more efficient and effective.

A comprehensive factorial design was utilized to investigate the combined impact of two independent variables: drug: phospholipids ratio (X_1, W/W) and temperature (X_1,°C). These variables were studied at three different levels, represented as -1 (low), 0 (middle), and +1 (high), resulting in a 3^2-factorial design with nine distinct combinations, as detailed in Table 1. The % Drug Entrapment Efficiency (Y_1) was considered as the response parameter for the study (Jain *et al*., 2019).

Evaluation of Phytosomal Formulation

% Entrapment efficiency

To assess the drug entrapment within the *Euphorbia hirta* phytosomes, a suspension containing 100mg of the phytosomes was prepared in 10 mL of chloroform. The mixture was then subjected to centrifugation at 25000rpm for 30 min to separate the phytosomes, capturing any un-entrapped drug in the supernatant. The free drug in the supernatant was dissolved in a mixture of Methanol and water, and its concentration was determined by measuring absorbance at 321nm using a UV-visible spectrophotometer (Said *et al*., 2002). The percentage of drug entrapment was calculated using a specific formula based on the experimental measurements.

$$\% EE = \frac{\text{quantity of drug added} - \text{quantity of free drug}}{\text{quantity of drug added}} \times 100 \text{ ---------- (1)}$$

Solubility test

To compare the solubility of the extract and phytosomes of *Euphorbia hirta* in water and hexane, separate experiments were conducted. In each experiment, 10 mL of water was added to an excess amount of the extract and an excess amount of the phytosomes, respectively. Similarly, 10 mL of hexane was added to an excess amount of the extract and an excess amount of the phytosomes. These mixtures were placed in sealed glass containers at room temperature (25°C). Each liquid mixture was agitated for 24 hr to allow for proper mixing. After the agitation period, the mixtures were subjected to centrifugation for 25 min at a high speed of 25000 rpm. This centrifugation process helped in separating the liquid components. Next, the supernatant, which is the liquid portion above the sediment, was collected and filtered through a membrane filter with a pore size of 0.45µ to remove any remaining particles or impurities. For the analysis, 1 mL of the filtered solution was taken and appropriately diluted. Then, the diluted solution was assayed using a UV-visible spectrophotometer, and the absorbance was measured at a wavelength of 321 nm (Jain *et al*., 2019).

Percentage Practical Yield

The practical yield percentage was calculated to determine the percent yield of phytosomes. The prepared phytosomes were collected and weighed to obtain the practical yield using the equation provided (Jain *et al*., 2019).

$$\%\text{Yield} = \text{Practical Yield/Theoretical Yield X } 100 \text{ -------- (2)}$$

Scanning Electron Microscope (SEM)

The surface characteristics of phytosomes and the Physical mixture will be analyzed using a SEM. For this analysis, powder samples of phytosomes and the physical mixture will be sprinkled onto circular aluminum stubs that have been pre-coated with silver glue to enhance electron conductivity. These stubs will then be placed inside the vacuum chamber of the SEM. The samples will be observed under the SEM at various magnifications, and micrographs will be recorded. A secondary electron detector will be utilized as a reference for the analysis (Sahu and Bothara, 2015).

Nuclear Magnetic Resonance (NMR)

The proton nuclear magnetic resonance spectra of phytosomes, soya lecithin, and *Euphorbia hirta* Extract were acquired using a Liquid State 500 MHz NMR instrument (Bruker Advance III HD NMR500 MHz). The NMR instrument allows for variable temperature settings, ranging from -150°C to +150°C, enabling a comprehensive analysis (Sahu and Bothara, 2015; Sharma and Sahu, 2016; Jain *et al*., 2019).

In vitro Drug release

The *in vitro* dissolution study of the prepared *Euphorbia hirta* phytosomes will be conducted using a dialysis method. To begin, a fresh suspension of the prepared *Euphorbia hirta* phytosomes, approximately 2 mL in volume, will be prepared. This suspension will then be added to a dialysis bag and securely tied. The dialysis bag containing the phytosomes will be suspended in a beaker filled with Phosphate buffer at pH 7.4. The contents in the beaker will be stirred at 50 rpm using a magnetic stirrer and maintained at a temperature of 37±1°C. At predetermined time intervals, 5 mL samples will be withdrawn from the beaker, and their release of *Euphorbia hirta* will be estimated by analyzing them using a UV-visible spectrophotometer at 321 nm (Sahu and Bothara, 2015; Sharma and Sahu, 2016; Jain *et al*., 2019).

Formulation of Capsule of *Euphorbia hirta* phytosomes

Precisely measured amounts of 100 mg *Euphorbia hirta* Extract and 200 mg Phytosome (equivalent to 100 mg extract) were taken. Subsequently, these quantities were carefully placed into a size #0 capsule shell using a manual capsule filling machine (Patel *et al.*, 2012).

Evaluation of Capsule

Disintegration test for capsules

Put one capsule into each of the six tubes of the basket. Then, hang the entire setup in water at a temperature of 37°C±2°C, with the basket being repeatedly dipped in the water 30 times per minute. The capsules will be considered successful in the test if there are no drug remnants, or any fragments of the capsule shell left on the number 10 mesh screen within the tubes (Patel *et al.*, 2012).

Weight variation test for capsules

A total of 10 capsules are randomly selected and weighed. The average weight of these capsules is determined, and then each individual capsule is weighed separately, with their individual weights recorded. To pass the test, each capsule's weight must be within 90-110% of the calculated average weight (Patel *et al.*, 2012).

In vitro Dissolution

Dissolution is utilized as a method to predict how quickly drugs are absorbed and their bioavailability when administered orally. In this process, a Paddle type dissolution apparatus containing 900 mL of Simulated Gastric Fluid (SGF) with a pH of 7.4 is placed in a jar, and the temperature is maintained at 37°C±2°C. The paddle in the apparatus rotates at a speed of 50 rpm for duration of 12 hr. At specific time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hr), 5 mL of the dissolution medium is withdrawn and replaced with fresh dissolution medium. The withdrawn samples are then analyzed using a UV Spectrophotometer set at 321 nm (Patel *et al.*, 2012).

RESULTS

Physico-chemical analysis of *Euphorbia hirta*

The *Euphorbia hirta* extracts was crystalline power with characteristic bitter taste.

Preliminary phytochemical screening of the extract

The medicinal properties of *Euphorbia hirta* extracts are believed to be attributed to several secondary metabolites found in them, including alkaloids, saponins, flavonoids, terpenoids, glycosides, tannins, and phenolic compounds. Conducting preliminary screening tests can be beneficial in identifying these bioactive components, leading to potential drug discovery and development. Additionally, these tests aid in quantifying

Table 1: 3^2 factorial design experimental trials.

Experimental trials	Drug: Phospholipids Concentration (X_1, W/W)	Temperature (X_2,°C)
F1	1.2	60
F2	1.2	50
F3	1.1	40
F4	1.3	60
F5	1.3	40
F6	1.1	50
F7	1.3	50
F8	1.2	40
F9	1.1	60

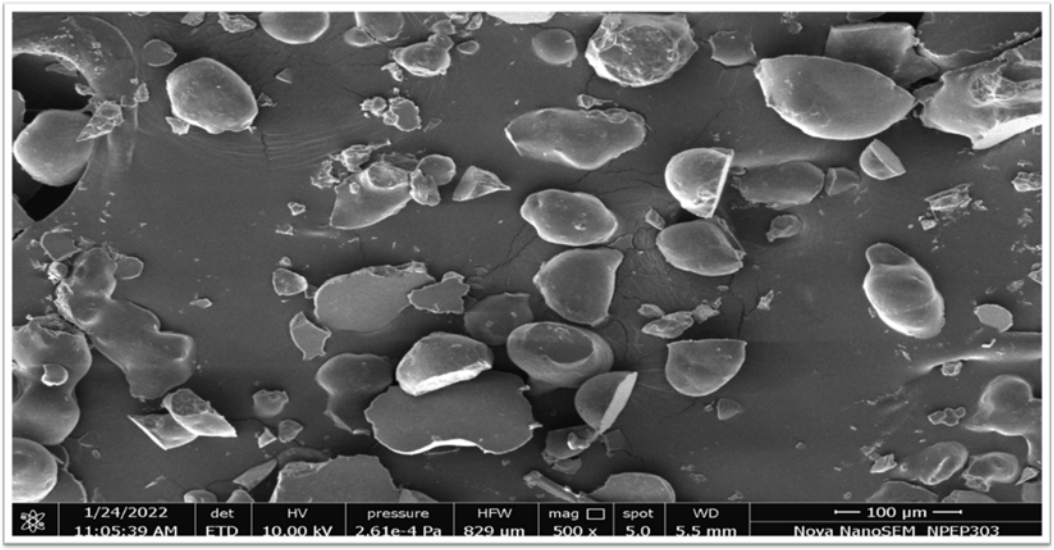

Figure 1: Scanning electron microscope.

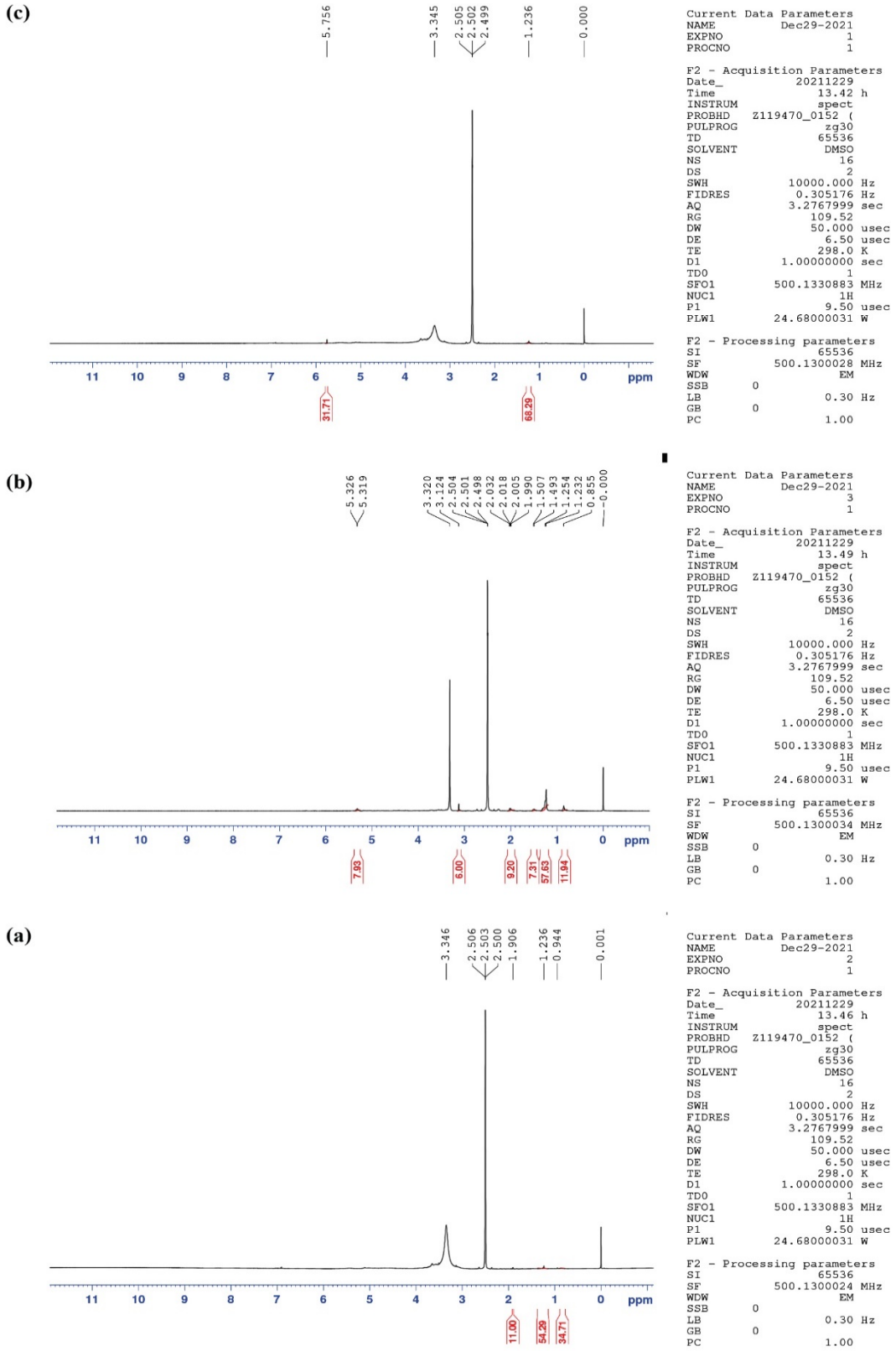

Figure 2: ^{1}H NMR of *Euphorbia hirta* extract (b) ^{1}H NMR of Soya lecithin (c) ^{1}H NMR of *Euphorbia hirta* phytosomes (F9).

and separating pharmacologically active chemical compounds (Amit *et al.*, 2007). After subjecting the *Euphorbia hirta* extract to various qualitative chemical tests during preliminary phytochemical screening, it was found to contain alkaloids, glycosides, flavonoids, saponins, and tannins. On the other hand, fats and oils and steroids were not present in the *Euphorbia hirta* extract.

Formulation design

Several formulation batches of polymeric nanoparticles were created using a 3^2-factorial design. The factors being studied were the ratio of *Euphorbia hirta* to phospholipid (X_1) and the reaction temperature (X_2), with their respective levels presented in Table 1. The response parameter or dependent variable in this study was the Entrapment Efficiency (%EE) (Y_1).

In the experimental trials conducted using a 3^2 full factorial design, the results of the entrapment efficiency (% yield) were obtained. These results indicated that the entrapment efficiency of the extract was significantly influenced by both variables under study: the *Euphorbia hirta* to phospholipid ratio (X_1 W: W) and the reaction temperature (X_2°C). Throughout the nine experimental batches examined, where different combinations of these variables were tested, the observed entrapment efficiency ranged from 66% to 96%. Based on the observations from these trials, the optimized batch (F9) was identified, which had an Extract: phospholipid ratio (X_1 W: W) of 1:1 and a reaction temperature (X_2 °C) of 60°C.

Evaluation of Phytosomal Formulation

% Entrapment efficiency

The % EE) is influenced by the characteristics of both the drug and the polymer employed. Out of all the batches tested, the thin film hydration method yielded the best % EE. The optimized batch, with a drug to polymer ratio of 1:1 and a temperature of 60°C, demonstrated a remarkable % EE of 95.89%.

Solubility Test

Solubility test was performed on both formulation of extracts and phystomes using different solvents. The solubility data was shown as in Table 2.

Percentage Practical Yield

The significant result of Percentage practical yield of *Euphorbia hirta* was found 95%.

Scanning Electron Microscope (SEM)

The SEM images of *Euphorbia hirta* phytosomes (F9) provide valuable insights into the surface morphology and solid-state properties, as depicted in Figure 1. These images clearly demonstrate that the *Euphorbia hirta* extract underwent a complete transformation into phytosomes, where the extract was entirely encapsulated by phospholipids. The smooth appearance of the particles indicates that the crystalline nature of the particles in the *Euphorbia hirta* extract disappeared, and the phytosomes took on an amorphous form. The slightly asymmetrical shape of the phytosomes can be attributed to the usage of the anti-solvent precipitation technique during the formulation process.

Nuclear Magnetic Resonance (NMR)

NMR is a highly effective tool for studying the micro-structural as well as chain arrangement of polymers including both solid and solution states. NMR is important as a methodology because NMR transmissions can be appointed to atomic nuclei anywhere along polymer structure as well as side chains. As shown in Figures 2, ^{1}H NMR of soya lecithin displayed δ 0.855 (12H, s), δ 1.232-1.254 (58H, s), δ 1.493-1.507 (9H, s), δ 3.124 (6H, s) and δ 5.319-5.326 (8H, s). ^{1}H NMR of *Euphorbia hirta* extract displayed δ 0.944 (35H, s), δ 1.236 (54H, s), δ 1.906 (11H). ^{1}H NMR of *Euphorbia hirta* phytosomes displayed δ 1.236 (68 H, s) and δ 5.756 (32H, s). The comparison drawn between 1 H NMR graphs of EHE and EHP indicated differentiation in the downfield region (δ>3) and up field region (δ<3). Additionally, 8H at δ 5.319-5.326 in ^{1}H NMR of soya lecithin appeared at δ 5.756 (32H) in 1 H NMR of *Euphorbia hirta* phytosomes also supported the development of hydrogen bonds.

In vitro Drug Release

Figure 3 presents a comparison of the dissolving behavior between EHE and EHP. At the end of the seven-hr period, the *in vitro* release results for EHE and EHP were approximately the same, at around 20%. However, as the test progressed, EHP exhibited a significantly higher drug release rate compared to EHE. After 10 hr, EHP's drug release reached 33%, almost double that of EHE, which was at 13%, indicating a considerable difference with a statistical significance (p<0.05). Subsequently, EHE's dissolution behavior showed a moderate increase, reaching approximately 14%. In contrast, EHP continued to display a substantial increase in dissolution, reaching 48% at the end of 12 hr, revealing a highly significant difference (p<0.001) between the two formulations.

Evaluation of Capsule

Disintegration Test

The Disintegration time was noted for the Capsule (Extract) as 7.47±0.45 min. The Disintegration time was noted for the capsule (Phytosomes) as 7.51±0.50 min.

Table 2: Solubility Study.

Formulation	Water (mg/mL)	Octanol (mg/mL)
Euphorbia hirta extract	15.28	2.966
Euphorbia hirta Phytosome (F9)	2.25	5.57

Weight Variation Test

From the results all capsules having a deviation of ±10%, which means that all capsules have uniform weights. It was concluded that, all results were found to be in acceptable limit as mentioned in Table 3.

In vitro dissolution

As depicted in Figure 4, The phytosomes contained in capsule (F9) exhibited a significant increase in dissolution, reaching 63.20% after 12 hr, indicating that it releases its contents within an acceptable timeframe for conventional dosage forms. Similarly, the extracts in capsule also showed a substantial increase in dissolution, reaching 41.40% after 12 hr, suggesting that it also releases its contents within a suitable duration for conventional dosage forms.

DISCUSSION

The primary objective of drug delivery systems is to deliver an effective amount of medication to the appropriate location within the body while also ensuring that the desired concentration of the drug in the bloodstream is reached and maintained for a specific duration (Kolimi *et al.*, 2023). Unfortunately, conventional dosage forms have certain drawbacks, such as incomplete drug release, limited time spent in the gastrointestinal tract, and significant degradation in the liver before reaching systemic circulation.

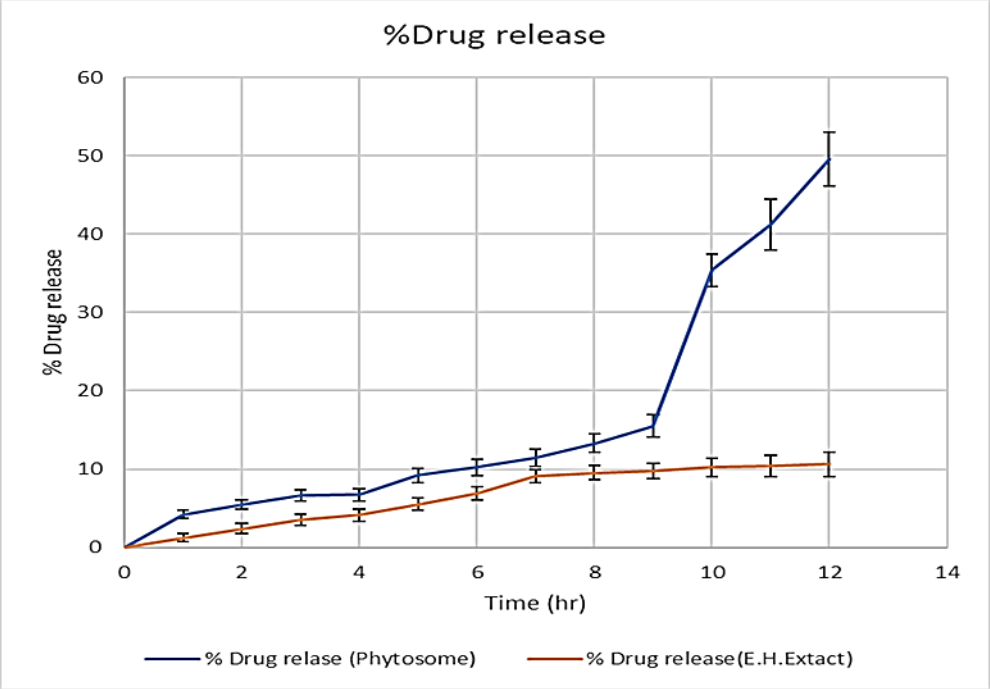

Figure 3: *In vitro* drug release of optimized batch and extracts

Table 3: Weight Variation Test.

	Extracts		Phytosomes (F9)	
Sl. No.	Weight (mg)	Deviation (%)	Weight (mg)	Deviation (%)
1	177	±0.8547	176	±1.3824
2	179	±1.9943	170	±2.0737
3	178	±1.4245	171	±1.4976
4	176	±0.2849	177	±1.9585
5	175	±0.2849	175	±0.8064
6	174	±0.8547	173	±0.3456
7	175	±0.2849	175	±0.8064
8	174	±0.8547	176	±1.3824
9	173	±1.4245	172	±0.9217
10	174	±0.8547	171	±1.4976
Average weight: 175.5 mg			Average weight: 173.6 mg	

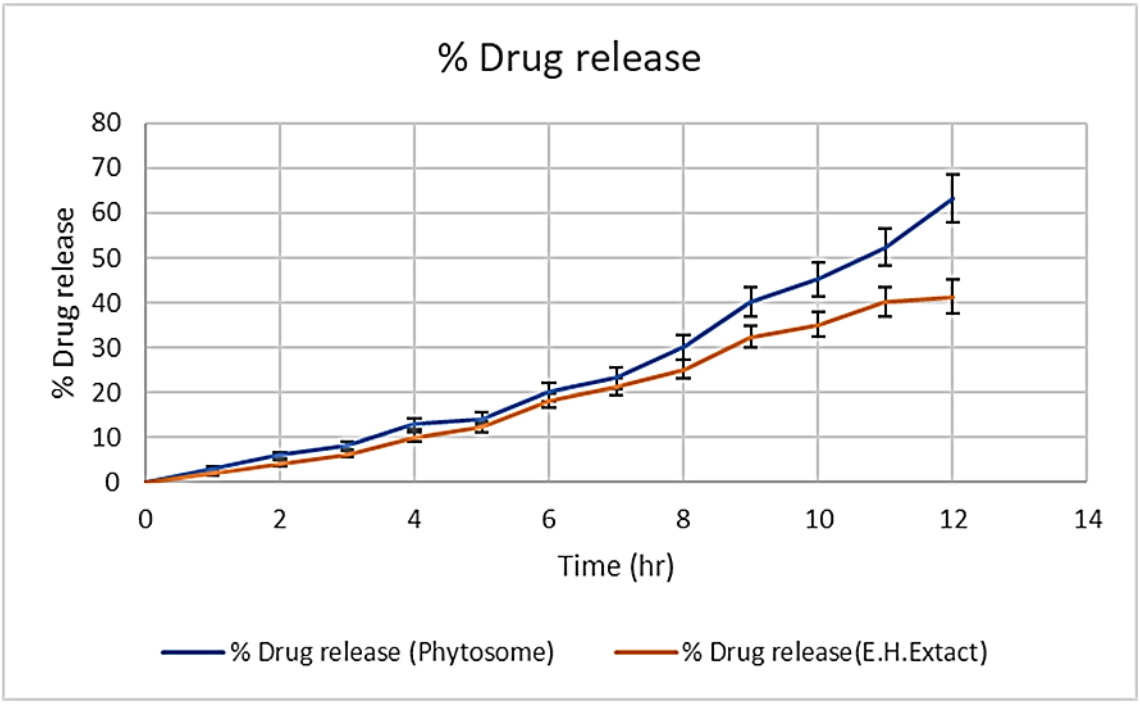

Figure 4: *In vitro* dissolution study of capsules.

Table 4: ***In vitro*** **dissolution test.**

Time (Hours)	% Drug release (Phytosomes in Capsule)	% Drug release (Extract in Capsule)
0	0	0
1	3.1±0.51	2.1±0.50
2	6.1±0.65	4.2±0.61
3	8.2±0.74	6.2±0.71
4	13.1±1.2	10.1±1.1
5	14.2±1.5	12.2±1.2
6	20.1±2.1	18.2±1.6
7	23.2±2.5	21.3±1.8
8	30.1±2.8	25.2±2.1
9	40.2±3.1	32.3±2.2
10	45.3±3.8	35.1±2.8
11	52.4±4.1	40.2±3.2
12	63.2±5.4	41.4±3.7

These issues result in lower bioavailability (Junnuthula *et al.*, 2022). To overcome these limitations, researchers have shifted their focus towards developing controlled and innovative drug delivery systems.

Among the different formulations tested, F9 exhibited the most favorable drug content and entrapment efficiency, with an impressive drug entrapment rate of 95.89%. The *in vitro* release studies demonstrated that the Phytosomes formulation, particularly F9, achieved the desired drug release of 49.60% over an extended period of 12 hr. Due to these promising results, F9 was selected as the optimal formulation and further encapsulated into capsules for additional evaluation.

Comparing it to an existing market product, the F9-based capsules demonstrated sustained release characteristics, releasing 63.20% of the drug over the same 12-hr period as depicted in Table 4. In summary, formulation F9 successfully transformed the raw extract of *Euphorbia hirta* into an innovative targeted drug delivery system known as Phytosomes. This novel system holds significant potential for effectively treating challenging medical conditions like UTI.

CONCLUSION

Among the various formulations tested, F9 displayed the most favorable drug content and entrapment efficiency, exhibiting an impressive drug entrapment rate of 95.89%. Through *in vitro* release studies, it was observed that the developed phytosomes formulation, especially F9, achieved the desired drug release of 49.60% over an extended duration of 12 hr. As a result, F9 was chosen as the optimal formulation and subsequently encapsulated into capsules for further evaluation. In comparison with an existing market product, the F9-based capsules demonstrated sustained release properties, with 63.20% of the drug being released over the same 12-hr period. In conclusion, formulation F9 successfully converted the raw extract of *Euphorbia hirta* into a novel targeted drug delivery system known as phytosomes. This

innovative system shows great potential in effectively treating medically challenging conditions such as UTI.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SEM: Scanning Electron Microscopy; **UTI:** Urinary Tract Infections; **% EE:** % Entrapment efficiency; **NMR:** Nuclear Magnetic Resonance; **EHE:** *Euphorbia hirta* Extract; **EHP:** *Euphorbia hirta* Phytosomes.

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