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Formulation and Evaluation of Phytosomes of *Thalictrum foliolosum* leaves extract

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ABSTRACT

Phytosome is a nanovesicle that combines plant extracts and phospholipids to produce more soluble fat complex and provide better absorption. *Thalictrum foliolosum* DC is widely distributed plant in the Himalayan region extending India, Nepal, Bhutan, South East Tibet and Burma between an altitude range of 1000-3400 m. Within India, it was recorded in Jammu & Kashmir, Himachal Pradesh, Punjab, Uttar Pradesh, Delhi, Sikkim, Arunachal Pradesh, Meghalaya, Bihar, Orissa, Andhra Pradesh and Tamil Nadu. In traditional medicine, various parts of this plant, including its bark, stems, and leaves, have been employed to address a range of health concerns, such as malaria, cancer, mental disorders, and pain relief. In the current days, the majority of the widespread diseases and nutritional disorders are treated with natural medicines. The efficiency of any herbal medication is reliant on the delivery of efficient level of the therapeutically active compound. But a harsh limitation exists in their bioavailability when administered orally or by topical applications. Phytosomes are lately introduced herbal formulations that are superior absorbed and as a result produced superior bioavailability and actions than the conformist phyto molecules or botanical extracts. The ethanolic extract of phytosome was prepared in soya lecithin. Soya lecithin has antioxidant activity. Evaluation of phytosomes for solubility study, entrapment efficiency, transition temperature, particle size and size distribution, optical microscopic study, zeta potential. The results showed the formation of a stable formulation with molecular interaction between the extract and phospholipids. The drug release from the phytosomes was sustained and showed significant improvement compared to the conventional extract.

Keywords: Phytosome, phospholipids, *Thalictrum foliolosum*, entrapment efficiency, etc.

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INTRODUCTION

The utilization of plants or their components in traditional medicine has a longstanding history, persisting from ancient times to the present day [1]. In the past century, there has been a significant focus on the chemical and pharmacological analysis of various plant extracts, aiming to understand their chemical composition and validate the indications of traditional medicine. However, it has been commonly observed that the isolation and purification of individual components from these extracts may result in a partial loss of their specific activity. To address this issue, Phytosomes, a patented technology developed by a leading manufacturer of drugs and Neutraceuticals, has emerged. This innovative approach involves integrating standardized plant extracts or water-soluble phytoconstituents into phospholipids, forming lipid-compatible molecular complexes known as phytosomes. The primary objective is to enhance the absorption and bioavailability of these components [2].

The Phytosomes process creates minute cells that serve as a protective barrier, shielding the valuable components of the herbal extract from degradation by digestive secretions and gut bacteria. Furthermore, Phytosomes exhibit improved capability to transition from hydrophilic environments to the lipid-friendly surroundings of enterocyte cell membranes, facilitating their passage into cells and ultimately reaching the bloodstream [3]. Notably, Phytosomes demonstrate superior pharmacokinetic and pharmacological parameters, making them favorable for use in managing acute and chronic liver diseases of toxic, metabolic, infective, or degenerative origins. Additionally, their applicability extends to various fields, including pharmaceutical and cosmetic compositions [4].

MATERIALS AND METHOD

Plant Material

Thalictrum foliosum leaves were collected from the outskirt area of Mandi, Himachal Pradesh. The plant was identified, authenticated, and certified (HIMCOSTE/HPSBB/5067) by Dr. Pankaj Sharma, Himachal Pradesh State Biodiversity Board, Shimla, India.

Preparation of Extracts

The process began with the preparation of plant leaves, which were initially cleansed with water to eliminate any dirt and foreign particles. After this cleaning step, the leaves were separated and subsequently subjected to shade drying. Once adequately dried, the leaves were milled to achieve a coarse powder texture, and then passed through a No. 14 sieve to obtain a consistent particle size. The resulting dried and powdered leaves of *T. foliolosum*, measuring 20 grams, were then placed within the tube of a Soxhlet apparatus in the form of a thimble. In this apparatus, they

were subjected to extraction using ethanol as solvent. This extraction process occurred at a temperature range of 60-65°C and lasted for approximately 3-4 hours. Subsequently, the extract obtained from this process, was filtered while still hot to remove any particulate matter, and they were then dried by evaporation using a rotary vacuum evaporator. The final dried extract samples were stored at low temperatures in a refrigerator for further analysis. Moreover, the residue left behind from each of the extractions was dissolved in the same solvent used for the initial extraction, preserving these residues for further analysis and investigation.

Formulation of phytosomes

Solvent evaporation technique

The specific amounts of *T. foliolosum* ethanolic extract and soya lecithin were refluxed with acetone at a temperature 50 – 60^{o}C for 2 h. The mixture was concentrated to obtain the precipitate which was filtered and collected. The dried precipitate of phytosome complex was placed in amber colored glass bottle and stored in refrigerator.

Table 1: Formula used for formulation of Phytosome

Batches	Extract (g)	Soya lecithin(g)	Cholesterol (g)	Temperature (^{o}C)	Methanol (ml)	DCM (ml)	Hexane (ml)
F1	5	5	1	40	20	10	20
F2	5	10	4	40	20	10	20
F3	5	7.5	1	40	20	10	20
F4	5	5	2.5	40	20	10	20
F5	5	10	2.5	40	20	10	20
F6	5	7.5	2.5	40	20	10	20
F7	5	5	4	40	20	10	20
F8	5	10	1	40	20	10	20
F9	5	7.5	4	40	20	10	20

Selection of the Optimized batch

An optimized formulation was selected based on the set criteria i.e. minimum particle size and maximum entrapment efficiency.

Characterization of Phytosomes

Entrapment efficiency

The entrapment efficiency of the Phytosomes was determined using the centrifugation technique. To do this, the Phytosome was first diluted with methanol and then subjected to centrifugation at 10,000 rpm for 30 minutes at a temperature of -4°C using a high-speed cooling centrifuge machine. After centrifugation, the supernatant was collected, and the quantity of free extract was assessed using a UV-visible spectrophotometer at wavelengths

of 328 nm and 366 nm [5,6]. The entrapment efficiency was calculated using the following formula:

$$= \frac{(\text{Total amt. of drug} - \text{amt. of free drug})}{\text{Total amt. of drug}} X\ 100 \ldots$$

Mean Particle Size and Size Distribution

Photon correlation spectroscopy (PCS) was utilized with the Zetasizer Nano ZS90 to determine the particle size and size distribution of the phytosomes. In this process, 20 µl of the phytosomal suspension was placed in a glass cuvette, and the programmed estimation mode with five runs was selected. The average particle size (z-average) and the size distribution of the prepared phytosomes were calculated based on the autocorrelation function of the intensity of light scattered from the particles, assuming a circular shape for the particles [221-223]. All measurements were conducted in triplicate and carried out at a temperature of 25°C. The particle size distribution was characterized using the span value, where a smaller span value indicates a narrower size distribution [7].

Soan = [D(90%)-D(10%)]/[D(50%)]

Where D (90), D (10) and D (50) are equivalent volume diameter at 90, 10 and 50% cumulative volume respectively.

Visualization

To visualize the phytosomes, scanning electron microscopy (SEM) was employed. SEM was used to determine the size distribution and surface morphology of the phytosome complex. The samples were prepared by sputter-coating them with a layer of gold/palladium for 120 seconds at 14 mA under an argon atmosphere. This coating was essential for secondary electron emission SEM imaging using a Hitachi-S 3400N SEM instrument. The morphology of the samples was observed at a voltage of 15.0 kV[8-10].

RESULTS AND DISCUSSION

Preparation and Optimization of Phytosome

The Phytosomes were prepared by solvent evaporation technique. The concentration of cholesterol and Soya Lecithin was optimized by preparing different trial batches. An optimized formulation was selected based on particle size, span value and entrapment efficiencyevaluation. Results obtained are given here in Table 2.

Table 2: Response parameters of phytosomal formulation of Ethanolic extract of *T. foliolosum*

Batch	Soya lecithin (g)	Cholesterol (g)	Particle size (nm)	Span(Y2)	Entrapment efficiency (%)
F1	5	1	143 ± 0.43	0.54 ± 0.04	45.48 ± 1.32
F2	10	4	198 ± 1.45	0.66 ± 0.05	52.72 ± 0.85
F3	7.5	1	220 ± 1.51	0.75 ± 0.65	58.69 ± 0.35
F4	5	2.5	255 ± 2.31	1.10 ± 0.23	63.53 ± 1.53
F5	10	2.5	295 ± 0.53	0.53 ± 0.14	82.43 ± 1.65
F6	7.5	2.5	312 ± 1.37	0.80 ± 0.53	77.34 ± 1.43
F7	5	4	343 ± 1.02	0.34 ± 0.58	79.58 ± 1.51
F8	10	1	397 ± 0.56	0.67 ± 0.74	73.34 ± 1.98
F9	7.5	4	456 ± 0.32	0.98 ± 0.32	76.59 ± 1.82

(Data are expressed as mean ± SD, n = 3)

For all the nine formulations particle size vary from 143 to 456 nm, span value 0.34 to 1.10 and entrapment efficiency from 45.48% to 82.43%. It was observed that mean particle size increased with increase in phospholipid concentration. This is due to the increase in number of the polymeric chains per volume unit of solvent that leads to collisions and formation of larger nanoparticles. Entrapment efficiency also increased as the extract/soya lecithin ratio was increased, which might be due to the increased polymer content which was expected to improve the entrapment efficiency byproviding more space to incorporate the drug.

Characterization

Usually, the characterization of phytosomes needs multiple techniques to certify and validate its size, form and morphology. The prepared optimized formulation of ethanolic extract of *T. foliolosum* was subjected to physicochemical and functional characterization. Physicochemical characterization includes determination of entrapment efficiency, visualization (structural studies), zeta potential measurement, DSC.

Entrapment efficiency

High speed cooling centrifuge machine was used to determine entrapment efficiency. Entrapment efficiency increased as the extract/soya lecithin ratio was increased, which might be due to the increased polymer content which was expected to improve the entrapment efficiency by providing more space to incorporate the drug. The entrapment efficiency of optimized formulation of EETF found to be(F5) 82.43%.

Mean particle size and size distribution

Table 2 depict the result of mean particle size and size distribution obtained by EETF. It was observed that mean particle size increased with increase in phospholipid concentration. This

is due to the increase in number of the polymeric chains per volume unit of solvent that leads to collisions and formation oflarger nanoparticles.

Visualization

Visualization was done by using Scanning Electron Microscope for determination of solid state properties and surface morphology of phytosome. SEM of optimized formulation is shown in Figure 1. The particle size was found to be 295 nm for EETF.

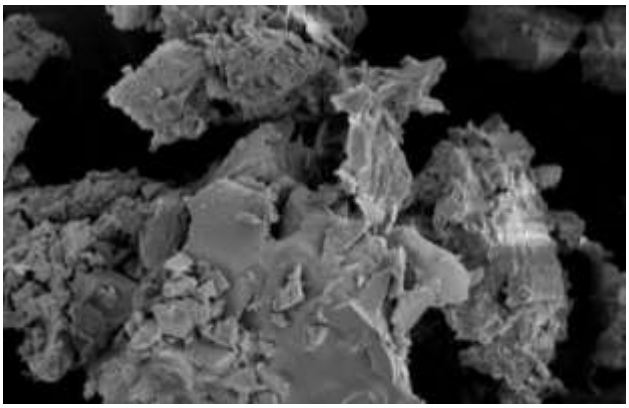

Figure 1: SEM of optimized formulation (F5) of EETF

Zeta potential

Zeta potential is identified with the charge on the surface of the molecule, thus impacts an extensive range of properties of colloidal materials, for example, their stability, interaction with electrolytes, and suspension rheology. Literature suggested that zeta potential value should lie in the range of -20 mv to +30 mv. Zeta potential was found to be -19.35mv for optimized formulation (F5) of EETF that indicates the formation of stable formulation.

DSC of optimized formulation (F5) of EETF

The thermogram of soya lecithin (Figure 2) gives two distinct peak at 86.71^{o}C and 202.91^{o}C. The first peak indicates melting while second one is attributed to transition temperature. The thermogram of cholesterol gives two peak at 41.87^{o}C and 103.76^{o}C and one sharp peak at 150.90^{o}C. This sharp peak indicates the melting point of cholesterol. The thermogram of *T. foliolosum* ethanolic extract give only one peak i.e 97.38^{o}C. Finally the thermos gram of optimized formulation of EETF gives peak two new peaks at 74.92^{o}C and 170.44^{o}C indicating the formation of new individual compound.

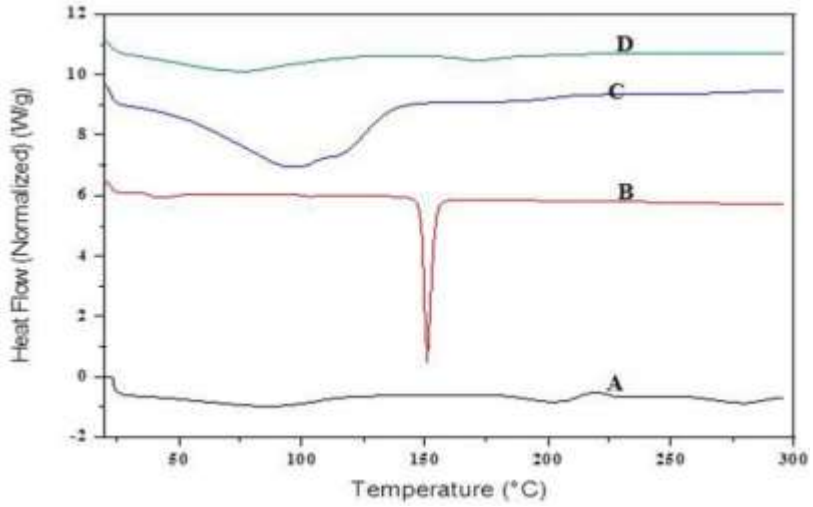

Figure 2: Comparative DSC thermogram of (A) Soya lecithin (B) Cholesterol (C) EEME (D) Optimized formulation (F5)

From the above study it can be concluded that a stable formulation is formed by some molecular interaction that can be van der Waals forces or hydrogen bonding between extract and phospholipids that distributed the extract molecularly into phospholipid. The study illustrates that phase transition takes place along with isomeric changes in the structure of phospholipid and reason behind this is movement of polar segment of phospholipid at increasing temperature.

CONCLUSION

The study aimed to develop and characterize the phytosomes for *T. foliolosim*, a medicinal The Soxhlet extraction process was used to prepare the plant extract, and phytochemical screening and physicochemical evaluation were conducted. Phytosomes were prepared using the solvent evaporation technique, and statistical analysis was performed to optimize particle size, span value, and entrapment efficiency. The optimized formulation (F5) of *T. foliolosum* leaves ethanolic extract underwent physicochemical and functional characterization, including in vitro release studies and in vivo anti-diabetic activity evaluation. The results showed the formation of a stable formulation with molecular interaction between the extract and phospholipids. The drug release from the phytosomes was sustained and showed significant improvement compared to the conventional extract.

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