Fabrication and Characterization of Herbal Liposomal Drug Delivery System

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Abstract
Herbal liposomes are innovative vesicular drug delivery methods intended to improve the therapeutic efficacy, stability, and bioavailability of herbal compounds. This study concentrates on the development and assessment of herbal liposomes incorporating specific plant extracts with possible pharmacological effects. Liposomes were synthesized with phospholipids and cholesterol through the thin film hydration technique, tuned for appropriate vesicle size, entrapment efficiency, and stability. The developed herbal liposomes were assessed for multiple physicochemical parameters, including appearance, vesicle size, zeta potential, pH, drug entrapment effectiveness, in vitro drug release, and stability investigations. Microscopic analysis verified the development of spherical vesicles with consistent distribution. The formulated preparations demonstrated adequate encapsulation efficiency and regulated release characteristics, signifying enhanced delivery of herbal active compounds. Subsequent assessment revealed improved stability and superior diffusion properties of the liposomal formulation relative to traditional herbal formulations. The findings indicate that liposomal encapsulation can markedly enhance the therapeutic efficacy of herbal medications by augmenting their solubility, permeability, and sustained release characteristics. In conclusion, the formulated herbal liposomal system demonstrated potential as an excellent carrier for herbal bioactive chemicals, enhancing both efficacy and patient adherence to herbal therapy.

Keywords: Liposomal Drug Delivery, Herbal Formulation, Phytosomes, Nanocarriers, Controlled Drug Release, Herbal Nanotechnology

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Introduction

The use of herbal medicines has gained popularity throughout the world in recent times. Often seen as natural alternatives with fewer side-effects than synthetic medicines, these medicinal plants and their phytochemical constituents are thought to have potential applications in the management of a wide range of health conditions. As such, many whole herbal preparations, herbal extracts and isolated phytocontituents have been subjected to pharmacological and clinical research, in which the in vitro demonstrated benefits of the phytochemical constituents do not always translate directly to in vivo and clinically demonstrated benefits [1]. Novel drug delivery systems have the potential to address some of these limitations. The distribution of both synthetic and natural drugs can be controlled by incorporation into a carrier system or by making an amendment to the structure of the drug, helping them to act longer and, more effectively within the body [2]. Novel drug delivery systems are a combination of pharmaceutics, bioconjugate chemistry, polymer science and molecular biology with unique physicochemical properties such as ultrasmall and controllable size, large surface area to mass ratio, high reactivity and functionalizable structure [3]. Some of the key drawbacks in traditional therapeutics can be overcome due to enhanced pharmacokinetic (solubility and bioavailability) and pharmacodynamic properties and the physical and chemical stability of nanoparticles. Nanoparticles also provide a means of sustained delivery and thus minimize the frequency of drug administration [4]. Nanoformulation is an emerging trend that has already yielded some interesting results in the development of novel phytochemical delivery systems [5]. Herbal medicines are commonly used today in the management of a wide variety of conditions ranging from dermatitis and psoriasis to skin infections to acne. However, the skin does provide a formidable barrier and novel drug delivery systems are being turned to as a means to improve transdermal absorption [6].

Topical/superficial disease caused by fungal pathogens Superficial fungal infections occur in the outermost layers of the skin, nails, hair, and mucous membranes. Dermatophytosis Dermatophyte fungi are organisms that digest keratin [7]. Medicinal plants are of great importance to the health of individuals and communities, and their importance lies in the chemical substances that produce a definite physiological action on the human body [8]. Many of the pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis, and quinine while their purification and quantification make them more predictable and chemical processing can sometimes modify their effects in desirable ways. Herbal remedies tend to

have a more complex and subtle mix of chemicals and can sometimes offer access to drugs or combinations of drugs that the pharmaceutical industry has not yet exploited. These natural compounds formed the basis of discovering modern drugs [8]. Fungal infections come in a wide variety, from superficial ones that affect the skin to systemic ones that invade interior organs. Traditional drug delivery methods, such as creams, lotions, ointments, gels, and liniments, have limited bioavailability. The various medicinal plants showed beneficial physiological activity on human beings. Opium, aspirin, digitalis, and quinine are just a few of the phytopharmaceuticals with a long history of treatment usage [9]. A novel drug delivery system uses a ground-breaking strategy to address the drawbacks of traditional drug delivery systems. Liposomes are spherical vesicles having an aqueous core enclosed by one or more phospholipid bilayers or lamellae. Liposomes are most frequently classified on the basis of their size (small, large and giant vesicles), number of bilayers (uni-, oligoand multi-lamellar) and phospholipid charge (neutral, anionic or cationic) [10]. The goal of the current study is to create a herbal liposomes that is safe, efficacious, and stable for topical treatment. The activities of liposomes as topical medication delivery systems to the skin vary based on their size, lipid and cholesterol composition, ingredient percentage, lamellarity, and surface charge. liposomes can overcome several barriers to cutaneous medication delivery, improve penetration through the stratum corneum, and reduce systemic effects through their localizing properties. Tinospora cordifolia commonly named as “Guduchi” in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude [11]. Ocimum sanctum L. (also known as Ocimum tenuiflorum, Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. Tulsi, the Queen of herbs, the legendary ‘Incomparable one’ of India, is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. The sacred basil, Tulsi, is renowned [12] for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East. Garlic (Allium sativum) is a species of bulbous flowering plant in the genus Allium [13]. Garlic is easy to grow and can be grown year-round in mild climates.

Material and methods

The plants *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* were collected from market or nursary.

Pharmacognostic study

In view of its diverse medicinal applications and in order to ensure the quality, authenticity and assay, and in view of lack of pharmacognostic study the present investigation was undertaken with an objective to evaluate *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* on various pharmacognostic parameters, such as macroscopic, physiochemical, and phytochemical studies of the plant. Fresh galls were taken for morphological and histological studies. Coarse powder was used to study the microscopic characters and physicochemical investigations. The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc.

Physicochemical analysis

Physicochemical values such as % of ash values and extractive values were determined according to the well-established protocols. The physicochemical analysis was investigated for the powder drug i.e. determination of ash, loss on drying, moisture content.

Extraction of plant material

The extracts of *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* was prepared and they are as-

Methanolic Extract

Coarsely powdered 100g *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* was macerated and extracted with 250 ml methanol at room temperature for 7 days and the extract was concentrated, frozen and lyophilized by lyophilizer.

Physical characterization of extract

Different physical parameters of extracts including their colour and percentage yield were obtained and extracts were weighed and percentage yields were calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Preliminary phytochemical screening

Phytochemical screening means to analyze the plant material for its chemical constituents. It involves the isolation of active constituents and their qualitative identification. Extracts of the selected plants were subjected to qualitative chemical test to assess the presence of alkaloid, glycosides, proteins, amino acids, steroids, tannins, carbohydrates, phenol compounds by using standard screening procedure.

Development of Liposomes

Multi-lamellar liposomes comprising extracts of Tinospora cordifolia (Giloy), Ocimum tenuiflorium (Tulsi), and Allium sativum (Garlic) were synthesized utilizing the thin film hydration process.

The plant extract, soya lecithin, and cholesterol were dissolved in a chloroform and methanol combination at a ratio of 9:1. The aforementioned solutions are transferred into the round-bottom flask of the rotary flash evaporator. In a rotary flash evaporator, the organic solvent evaporates at 60ºC for 15 minutes at 90 RPM. After the evaporation of the organic solvent, a thin layer forms on the inner surface of the round-bottom flask. The thin layers were dried overnight in a vacuum oven. The thin lipid layer suspension in phosphate-buffered saline (PBS) at pH 7.4 was vortexed for 10 minutes and subsequently hydrated for 1 hour at 70ºC with a rotation speed of 90 rpm. The liposomal suspension is subjected to ultra-centrifugation at 3000 rpm for 30 minutes. Subsequently, centrifuge the settled liposome in PBS. The suspension of liposomes is sonicated for 15 minutes at 65ºC to produce small unilamellar vesicles (SUV). In this investigation, four batches were made, and their formulas and compositions are presented.

Table 1: Composition of plant extract containing liposomes

S. No.	F. Code	Tinospora cordifolia (Giloy)	Oscimum tenuiflorium (Tulsi)	Allium Sativum (Garlic)	Soya Lecithin	Cholesterol
1	L 1	150 mg	0	0	100 mg	15 mg
2	L 2	0	150 mg	0	100 mg	15 mg
3	L 3	0	0	150 mg	100 mg	15 mg
4	L 4	50 mg	50 mg	50 mg	100 mg	15 mg
5	L 5	75 mg	75 mg	0	100 mg	15 mg
6	L 6	0	75 mg	75 mg	100 mg	15 mg
7	L 7	75 mg	0	75 mg	100 mg	15 mg

Characterization of Liposomal Drug Delivery System:

Particle size

The particle size of liposome is generally taken by zeta sizer instrument. This instrument containing Malvern PCS software. Before taking the result of sample solution the sample must be diluted with distilled water. The distilled water not interferes with result. Then after dilution the result were taken. The particle size must be required in nano range some time it goes to micron range if multilamellar vesicles are present. This software was taken the average particle size of liposome. The particle size of sample solution was determined by using light scattering technique and by transmission electron microscope. If the particle size of liposome increases then decrease the uptake and bioavailability of drug. The analysis of particle size was carried out for 60s at 165oc scattering angle of detection. The particle size is most important, the particle size of liposome in nano range are having more effective drug delivery as compare to micron range. The one advantage of large particle size liposome is having more area to fill more drug but it has very slow-release pattern. Various method is used.

PDI

PDI is also called “particle size distribution”. If the sample having very broad size distribution, then poly dispersed value goes to more than 0.7. The PDI of liposome is also obtained by photon Correlation spectroscopic analysis. During formulation of liposome the effort of manufacturer are must be to achieve lowest PDI value.

Zeta Potential

The zeta potential means the charges which are present on the surface of liposome. The many times the charge is present on the surface of liposome. This charge is come due to the component or ingredient which was used during the manufacturing. Some charge is must be required on surface of all liposome present in formulation, due to some charge all liposome particle repeal to each other and coagulation of particle are avoided. The zeta potential of liposome was taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25ºc with the angle of detection 90o. The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of liposomal particle.

Entrapment Efficiency

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer at 221 nm as gallic acid content determination. A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the liposome.

%Drug Entrapment = (W-w/W) × 100

Result and discussion

Tinospora cordifolia (Willd.) Miers, (Guduchi) is an evergreen perennial climber belongs to the family Menispermaceae. It is a plant of significant medicinal importance in the indigenous systems of medicine

and designated as Rasayana. All the parts of this plant are reported for various ethnobotanical and therapeutic uses. Tulsi is an aromatic shrub in the basil family Lamiaceae (tribe ocimeae) that is thought to have originated in north central India and now grows native throughout the eastern world tropics. Allium sativum is a perennial flowering plant growing from a bulb. The leaf blade is flat, linear, solid, and approximately wide, with an acute apex. The plant may produce pink to purple flowers.

Table 2: Macroscopic characteristics of Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)

Characteristics		
Tinospora cordifolia	Shape	heart-shaped leaves
	Surface	Smooth
	Colour	green
	Odour	characteristic
	Taste	Bitter
Oscimum tenuiflorium	Shape	Mixture of coarse and fine
	Surface	Smooth
	Colour	green
	Odour	spicy aroma
	Taste	astringent and bitter flavor
Allium Sativum	Shape	Mixture of coarse and fine
	Surface	Smooth
	Colour	Creamish white
	Odour	Strong
	Taste	pungent

Physiochemical constants

The percentage of total ash, acid insoluble ash, sulphated ash and water-soluble ash in given table 3. The ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The loss on drying and foreign matter was 9.50 and 0.10 respectively. The extractive values are primarily useful for the determination of exhausted drugs.

Table 3: Physiochemical parameters of *Tinospora cordifolia (Giloy, Guduchi), Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)*

Parameters	***Tinospora cordifolia* (Giloy)**	***Oscimum tenuiflorium* (Tulsi)**	***Allium Sativum* (Garlic)**
Foreign matter (% w/w)	0.1	0.9	1.2
Loss on drying (% w/w)	9.5	8.7	9.8
Total ash value (% w/w)	5.02	4.53	5.35
Acid insoluble ash (% w/w)	1.51	2.31	1.97
Water soluble ash (% w/w)	3.22	2.49	3.05
Sulphated ash (% w/w)	0.21	0.35	0.98

Extraction of plant material

The yield of methanolic extract of *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* was shown in Table 4. Methanolic extract of all plants was in semi-solid form. Note – 100 g of crude plant material was taken for extraction, The extract was in semisolid- solid form. The extract was stored in tightly packed container

Table 4: Physical characterization of extract

S. No.	**Methanolic Extracts**	**Extraction Time (days)**	**Colour**	**% Yield**
1	*Tinospora cordifolia (Giloy)*	7	Green	19.68
2	*Oscimum tenuiflorium (Tulsi)*	7	Dark green	20.23
3	*Allium Sativum (Garlic)*	7	Brownish	18.71

Preliminary phytochemical analysis

Investigations on the preliminary phytochemical screening of *Tinospora cordifolia (Giloy), Oscimum tenuiflorium (Tulsi)*, *Allium Sativum (Garlic)* extracts revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids and carbohydrates in methanolic and aqueous extracts respectively (Table 5).

Table 5: Preliminary phytochemical screening of plants

Chemical constituents	***Tinospora cordifolia (Giloy)***	***Oscimum tenuifloriu m (Tulsi)***	***Allium Sativu m (Garlic)***
Phenols	+	+	+

Flavonoids	-	+	+
Steroids	+	-	-
Triterpenes	-	-	-
Tannins	+	+	+
Saponins	+	-	-
Alkaloids	+	+	+
Glycosides	+	-	-
Carbohydrates	+	+	+

+ Denotes the presence of the respective class of compounds.

Characterization of Liposomal Drug Delivery System

Seven liposomal formulations containing different combinations of Tinospora cordifolia (Giloy), Ocimum tenuiflorium (Tulsi), and Allium sativum (Garlic) were prepared using soya lecithin and cholesterol as lipid components. Each formulation contained a fixed lipid ratio (Soya lecithin 100 mg and cholesterol 15 mg), while the herbal drug combinations were varied to evaluate the effect of composition on liposomal performance.

Table 6: Various characterization of liposomal drug delivery system

S. No	F. Code	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment Efficiency (%)
1	L1	182.4 ± 3.8	0.328 ± 0.02	−26.7 ± 1.3	68.4 ± 2.1
2	L2	176.2 ± 3.1	0.312 ± 0.03	−27.9 ± 1.4	72.3 ± 2.1
3	L3	168.5 ± 2.9	0.298 ± 0.02	−29.4 ± 1.2	70.8 ± 2.3
4	L4	142.6 ± 3.4	0.241 ± 0.01	−34.8 ± 1.5	88.7 ± 2.6
5	L5	155.7 ± 3.3	0.256 ± 0.02	−31.8 ± 1.6	82.5 ± 2.5
6	L6	198.3 ± 4.1	0.341 ± 0.03	−24.3 ± 1.2	85.9 ± 2.2
7	L7	142.6 ± 3.4	0.214 ± 0.02	−34.8 ± 1.5	84.6 ± 2.4

All formulations were successfully prepared using thin film hydration method. Lipid concentration remained constant ensures valid comparison. Variation in herbal composition allowed evaluation of single herb effect with dual synergistic effect, such expected performance ranking. Hence, L4 is optimized formulation due to broad therapeutic spectrum with bbalanced phytochemical loading and synergistic activity of all three herbal extracts.

Seven liposomal formulations (L1–L7) were successfully designed using varying proportions of herbal extracts while keeping the phospholipid (soya lecithin) and cholesterol concentration constant (100 mg and 15 mg respectively). The variation in herbal drug loading allowed comparative evaluation of single-drug and combination formulations. L1 contained only *Tinospora cordifolia* (150 mg), L2 contained only *Ocimum tenuiflorium* (150 mg), L3 contained only *Allium sativum* (150 mg). These batches were prepared to evaluate the individual contribution of each herbal drug in liposomal form, L4 contained equal quantities of all three extracts (50 mg each). This formulation was designed to study the synergistic effect of combined herbal drugs, L5: *Tinospora cordifolia* (75 mg) + *Ocimum tenuiflorium* (75 mg), L6: *Ocimum tenuiflorium* (75 mg) + *Allium sativum* (75 mg), L7: *Tinospora cordifolia* (75 mg) + *Allium sativum* (75 mg). These batches were formulated to investigate the effect of dual-herb combinations.

All formulations maintained a constant lipid matrix composition (100 mg soya lecithin and 15 mg cholesterol), ensuring that any variation in performance can be attributed primarily to differences in herbal drug loading and combinations. The design provided a systematic platform to compare:

- Individual vs combined herbal effects
- Synergistic activity among herbal drugs
- Influence of herbal ratio on liposomal formulation performance

This formulation strategy enabled selection of an optimized herbal liposomal formulation for further physicochemical and biological evaluation.

The prepared nanogel formulations (L1–L7) were evaluated for particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency (EE%). These parameters are critical for determining the stability, uniformity, and drug-loading capability of nanosystems.

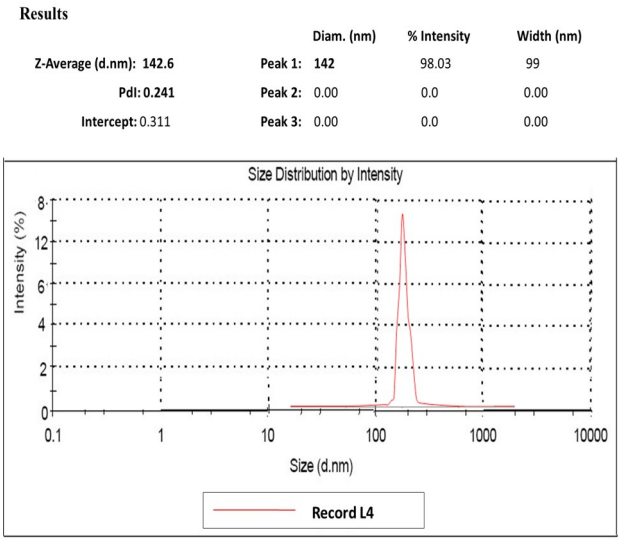

Figure 1: Particle Size characterization of liposomal drug delivery system (L4)

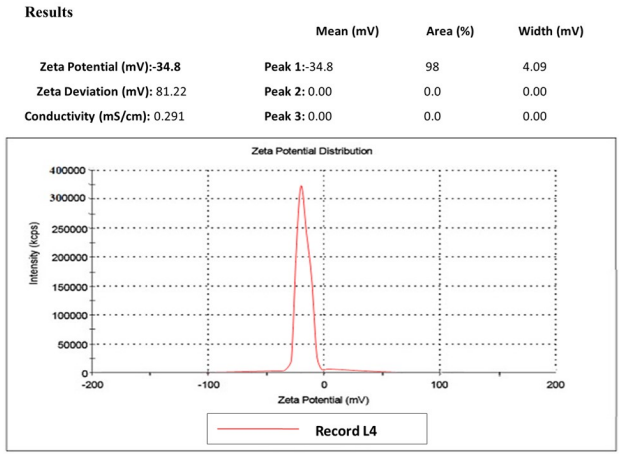

Figure 2: Zeta Potential characterization of liposomal drug delivery system (L4)

Conclusion

The present study successfully developed and characterized a herbal liposomal drug delivery system containing methanolic extracts of Tinospora cordifolia, Ocimum tenuiflorium, and Allium sativum. Preliminary phytochemical screening revealed the presence of several bioactive constituents responsible for antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory activities. These findings support the therapeutic significance of the selected herbal extracts and justify their incorporation into a novel liposomal delivery system. Seven liposomal formulations (L1–L7) were successfully prepared using the thin film hydration method. The formulations showed satisfactory physicochemical characteristics with particle sizes ranging indicating formation of nanosized vesicular systems suitable for enhanced drug delivery. The PDI values demonstrated good uniformity and homogenous distribution of vesicles, while negative zeta potential values confirmed adequate stability of the liposomal systems. Among all formulations, L4 exhibited the most attributed to the synergistic combination of all three herbal extracts in balanced proportion, leading to better phytochemical loading and enhanced stability of the liposomal vesicles. Overall, the study demonstrated that herbal liposomal formulations can serve as an effective nanocarrier system for improving stability, entrapment, and therapeutic potential of herbal bioactive compounds. The optimized formulation (L4) may provide enhanced biological activity and can be considered a promising candidate for further pharmaceutical and therapeutic applications.

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