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Review articles

Review Article: Preparation Methods, Physicochemical Characteristics, and Biological Performance of Herbal Extract-Loaded Liposomes

ABSTRACT

Liposomes are widely used to improve solubility, stability, release and delivery of bioactive compounds from herbal extracts. Liposome performance does not depend solely on one method of preparation. This review covers the relationships between liposome formation methods, post-processing techniques, extract characteristics, physicochemical properties and biological responses. The most common method for liposome preparation is thin film hydration because of its simplicity and flexibility, but the method generally yields large multilamellar vesicles prior to sonication or extrusion. Reverse-phase evaporation and polyol dilution can result in larger aqueous compartments that can help encapsulate hydrophilic extracts. Ethanol injection leads to rapid vesicle formation, and proliposome and Mozafari methods can be used for storage stability or to minimize the usage of organic solvents. Comparative studies indicate that sonication can produce particles with sizes ranging from 92 to 162 nm, but the smallest size does not necessarily correspond to the highest encapsulation efficiency. Encapsulation efficiency is formulation-dependent and has been reported to be anywhere from approximately 17% to nearly 100%, with most optimized formulations in the 60-90% range. The variation is mainly attributed to the polarity of the extract, the ratio of extract to lipid, the composition of phospholipid and sterol, solvents, and the methods of analysis. Encapsulation in liposomes generally improves the stability of antioxidants, anti-inflammatory activity, wound healing, anticancer cytotoxicity, antimicrobial activity and systemic exposure. However, these improvements are specific to the model and do not necessarily increase linearly with particle size or encapsulation efficiency. Therefore, the choice of method must be determined by the compatibility of the extract's characteristics, the desired vesicles' architecture, the route of administration, and the biological targets.

Keywords: *herbal extract; liposomes; preparation methods; encapsulation efficiency; biological activity*

INTRODUCTION

Herbal extracts are complex mixtures of compounds that differ in polarity, molecular size and stability. Such diversity allows a broad spectrum of biological activities but complicates the design of formulations. hydrophilic components may be quickly degraded or eliminated rapidly, lipophilic components tend to have low water solubility and poor bioavailability. Light, oxygen, temperature, gastrointestinal pH and inter-component interactions can also alter marker compound levels and activities. Thus, the success of extracts in chemical assays is not always translated into enough biological exposure at target tissues.

Liposomes have a dual-compartment structure which is suitable for herbal mixtures. The inner aqueous phase can contain hydrophilic components, and the phospholipid bilayer can accommodate lipophilic and amphiphilic components. The structure can protect active compounds, sustain release, improve dispersion in biological media and prolong contact with cells or tissues. Such improvements have been reported for extracts of green tea, silymarin, ginger, Centella asiatica, Hypericum perforatum, and other extracts [1–4].

Herbal extract liposomes are prepared by a highly diverse set of methods but the basic principles are the same. Techniques such as thin-film hydration, ethanol injection, reverse-phase evaporation, polyol dilution, proliposomes and the Mozafari method create different routes for vesicle formation. Particle size reduction or narrowing of size distribution is then often accomplished by sonication, extrusion and microfluidization. It is important to distinguish between primary formation methods and post-processing techniques as both influence vesicle architecture via different mechanisms [5,6].

Cross-study comparison also poses fundamental challenges. Two formulations prepared by the same method may present significantly different sizes, polydispersity indices, zeta potentials, and encapsulation

efficiency due to the differences in the ratios of extract to lipid, phospholipid compositions, cholesterol contents, solvent types, and levels of extract fractionation. The way encapsulation efficiency is calculated using total polyphenols versus a single marker compound can result in different interpretations [7,8]. The present review discusses the main methods of preparing liposomes loaded with herbal extracts, the effect of post-processing on the properties of vesicles and the correlation between physicochemical properties and biological performance. The analytical focus is on comparative evidence and mechanisms explaining variation in outcomes so as to avoid the reduction of method selection to a single technique assumed to be better for all extracts.

Principles of Herbal Extract Encapsulation in Liposomes

Liposome Structure and Distribution of Extract Components

Liposomes consist of one or more phospholipid bilayers encapsulating aqueous compartments. Highly hydrophilic compounds are primarily partitioned into the aqueous phase, lipophilic compounds are associated with the acyl chains in the bilayer, and amphiphilic molecules are situated at the lipid-water interface. This distribution determines the amount of aqueous phase, the surface area of the bilayer, the membrane fluidity and the strength of the interactions between the components of the extract and the phospholipids.

Crude extracts can not be regarded as a single active molecule. Polyphenols, saponins, alkaloids, terpenoids, sugars, proteins, and organic acids can either compete for the liposomal compartments or interact with the polar headgroups of the phospholipids. For the Orthosiphon stamineus extract, the total efficiency is not representative for all components, because marker compounds with higher and lower polarities show different encapsulation efficiencies [7]. Thus, the parameters of payload localization and characterization have to be interpreted depending on the composition of the extract.

Key Formulation Variables

The type of phospholipid affects the transition temperature, fluidity, permeability and oxidative stability of the bilayer. Phospholipids with unsaturated chains tend to produce more fluid membranes that allow easier incorporation of lipophilic components but are more susceptible to oxidation. Hydrogenated phospholipids increase rigidity and stability but may alter drug release and cell interactions. The effect of lipids composition on size, encapsulation and antitumor response was studied comparing unsaturated and hydrogenated lipids in liposomes prepared from goji berry extract by the same ethanol injection method [9].

Cholesterol or other sterols intercalate into phospholipids and regulate membrane packing. Leakage can be reduced and vesicle stability kept at appropriate levels during storage. If the level is too high the bilayer will be too rigid to allow the intercalation of components of the extract and it will be difficult to reduce the size. This is not the universal answer. An increase in proportion of cholesterol was associated with high encapsulation efficiency in Coleus (Coleus atropurpureus) leaf extract, whereas high sterol content reduced polyphenol encapsulation in wild thyme extract [10,11].

The liposomal compartment has a capacity, which is determined by the ratio extract/lipid. The total amount of encapsulated substance can be increased to a certain point by increasing the extract payload. But if the payload exceeds the capacity of the vesicles, the free extract fraction increases and the percentage of the encapsulation efficiency decreases. This pattern has been observed in *Asparagus racemosus, Piper longum*, ginseng and green tea [6,12–14].

Preparation Methods for Herbal Extract-Loaded Liposomes

Thin-Film Hydration

Thin film hydration is the most common method. Phospholipids, cholesterol and lipophilic components are dissolved in organic solvents which are then evaporated to form thin lipid film. The film is hydrated with an aqueous phase that may contain hydrophilic extract components. The hydration step produces multilamellar vesicles of high heterogeneity and relatively large size, which usually require an additional sonication, extrusion or homogenization. The strength of it is in the simple procedure, compositional flexibility and convenience for laboratory scale research. Limitations include the use of organic solvents, the possibility of non-homogeneous film formation, batch to batch variation and the need for post-processing. And solvent selection is important too. In the case of *Orthosiphon stamineus*, methanol with chloroform produced a more homogeneous film than ethanol-chloroform systems since the evaporation rates were matched, improving the extract dispersion after hydration [7].

Thin film hydration with size reduction can yield vesicles in the size range of about 95–200 nm with encapsulation efficiencies of 70–90% and there are many optimized formulations that show this. Examples are *Piper longum*, *Platanus orientalis*, *Glycyrrhiza glabra* and *Centella asiatica* [4,13,15,16]. These results show that the term thin-film hydration alone is not enough to predict the final product, hydration intensity, lipid composition and post-processing parameters should be reported together.

Ethanol Injection and Solvent Injection

In ethanol injection the lipids are dissolved in ethanol and then added in a controlled manner to an aqueous phase. Ethanol dilution reduces lipid solubility, leading to self-assembly of bilayers into vesicles. The method is relatively fast and does not require the formation of dry film and has better scalability. The results are sensitive to the injection rate, the organic to aqueous phase ratio, the lipid concentration, the temperature and the removal of residual ethanol.

Ethanol injection has been used for extracts of clove, black cumin seed, *Hypericum perforatum*, ginseng and goji berry [9,14,17–19]. Zhang et al. (2025) obtained ginseng extract liposomes of approximately 177 nm with a zeta potential of -24.6 mV and the encapsulation efficiency varied depending on the amount of payload [14]. This finding supports the fact that the same method still requires optimization of the component ratio.

Reverse-Phase Evaporation

Reverse-phase evaporation forms a water-in-organic emulsion containing lipids. Gradual solvent removal causes lipid layers to surround the aqueous phase, forming vesicles with relatively large internal aqueous compartments. This characteristic is advantageous for hydrophilic extracts; however, solvent exposure and pressure during evaporation may affect the stability of sensitive compounds.

The clearest evidence comes from direct comparisons on *Asparagus racemosus* root extract. Reverse-phase evaporation yielded oligolamellar vesicles of approximately 197–240 nm, smaller than the multilamellar vesicles obtained from thin-film hydration (328–457 nm). Its encapsulation efficiency reached 56.21% at the optimal extract-to-lipid ratio, slightly higher than thin-film hydration, though still lower than polyol dilution [12].

Polyol Dilution

The polyol dilution method disperses lipids in a polyol, followed by vesicle formation via controlled dilution with an aqueous phase. This method can produce oligolamellar vesicles with adequate aqueous volume without dry film formation. For *A. racemosus* extract, polyol dilution yielded sizes of 202–248 nm, zeta potentials of -12 to -19 mV, and a maximum encapsulation efficiency of 67.68%, the highest among the three evaluated methods [12]. Therdphapiyanak et al. (2013) also reported superior performance of polyol dilution over several other approaches regarding tyrosinase inhibitory activity of the same plant extract [20].

Proliposom Method

Proliposomes are dry or concentrated precursors that can be converted into liposomes upon hydration. Precursor states offer better storage convenience and fewer vesicle changes during liquid storage. This method is applied to rosehip, carob, *Momordica charantia* and wild thyme extracts. Jovanović et al. (2023) reported encapsulation efficiencies above 90% for the liposomes of rosehip extract and they retained their properties after ultraviolet exposure [21]. However, the quality of the final product strongly depends on hydration protocols, sterol composition and post formation processes.

Mozafari Method

In the Mozafari method, heating and stirring is employed to make vesicles without the use of conventional dry film procedures or detergents . This method is attractive for extracts with simple processing and low organic solvent residues. The best formulation of green tea extract presented a size of 419 nm, a polydispersity index of 0.28, zeta potential of −59.7 mV and encapsulation efficiency of 51.34% [6]. The vesicle size of rosemary extract was increased up to 583 nm with an encapsulation efficiency of 54.59% [22]. The relatively large size is a compromise to avoid high mechanical energy input.

Microfluidization and Hybrid Approaches

Microfluidization forces dispersions through narrow channels at high pressure, generating strong shear forces and micro-collisions. This method results in smaller size and better homogeneity, but is more equipment and energy consuming. After thin-film hydration, the black rosehip extract liposomes [23]. Dağ and Oztop (2017) also observed that microfluidization provided a better storage stability for green tea extract than ultrasonication in their test conditions [24].

Hybrid techniques incorporate primary vesicle creation with two or more phases of size reduction or coating. Su et al. (2024) synthesized phenylethanoside liposomes using ethanol injection, extrusion and ultrasonication. This combination improved encapsulation efficiency by about 8% over extrusion alone and 18% over ultrasonication alone [25]. The benefits of hybrid methods should be balanced against process complexity, repeatability and risk of payload degradation from cumulative energy input.

Tabel 1. Main preparation methods for herbal extract-loaded liposomes

Method	**Formation principle**	**General characteristics**	**Main advantages**	**Main limitations**	**Reference(s)**
Thin-film hydration	Formation of a dry lipid	Initially produces	Simple, flexible, and	Requires organic	Aisha et al., 2014; Akhlaghi et al.,

	film followed by hydration with an aqueous phase	relatively large and multilamellar vesicles	widely established	solvents and commonly requires additional size-reduction processes	2022; Demirel et al., 2025
Ethanol injection	An ethanolic lipid phase is injected into an aqueous phase	Vesicles form spontaneously and may have relatively small particle sizes	Rapid, simple, and potentially scalable	Sensitive to the organic-to-aqueous phase ratio and may leave residual ethanol	Sebaaly et al., 2015; Altıntaş and Erdoğmuş, 2023; Zhang et al., 2025; Peixoto et al., 2026
Reverse-phase evaporation	Formation of a water-in-organic emulsion followed by gradual solvent removal	Produces vesicles with a relatively large aqueous compartment	Suitable for hydrophilic extracts	Involves organic-solvent exposure and possible stress during evaporation	Plangsombat et al., 2016
Polyol dilution	Dispersion of lipids in a polyol followed by controlled dilution with an aqueous phase	Tends to produce oligolamellar vesicles	May provide relatively high encapsulation of hydrophilic constituents	Less widely used and less extensively validated for scale-up	Therdphapiyanak et al., 2013; Plangsombat et al., 2016
Proliposome method	A dry or concentrated lipid precursor is converted into liposomes upon hydration	Final vesicle properties depend strongly on the hydration process	More practical precursor storage and potentially improved stability	Final product quality is highly dependent on rehydration conditions	Jovanović et al., 2023; Jovanović et al., 2025a
Mozafari method	Heating and stirring without	Produces relatively stable but often larger	Reduces or avoids the use of	Limited particle-size control	Jahanfar et al., 2020; Jahanfar et al., 2021

	conventional dry-film formation	vesicles	conventional organic solvents	without additional post-processing	
Microfluidization	Application of high-pressure shear, turbulence, and collision forces	Produces small and relatively homogeneous vesicles	Good particle-size control and process reproducibility	Requires specialized equipment and relative	Dağ and Oztop, 2017; Su et al., 2024

Role of Post-Processing Techniques

Sonication

Sonication utilizes cavitation and shear forces to reduce the size of large vesicles to smaller ones. Probe sonication is more powerful than bath sonication and is often used after thin-film hydration. Sonication of rosemary oil liposomes produced sizes of 92–162 nm and polydispersity indices of 0.12–0.35, much smaller than those of thin-film hydration products without size reduction [26]. However, excessive energy can increase temperature, oxidize lipids, degrade herbal constituents or leach. To obtain effective size reduction without thermal degradation *Glycyrrhiza glabra* was processed using pulsed protocols (e.g. 10 seconds active, 15 seconds pause at 60% amplitude) [15].

Extrusion

Extrusion forces dispersions through membranes of defined pore sizes. The technique allows direct size control and narrower distributions, although repeated passes may reduce payload due to membrane deformation and leakage. *C. asiatica* liposomes extrusion through 100 nm membranes 120.6 ± 0.18 4 Under optimal condition [4]. Under optimal condition, *H. perforatum* formulations also achieved a PDI < 0.25 and encapsulation efficiency of 91.83 % by sequential extrusion through 400 nm and 200 nm membranes [3].

Homogenization and Microfluidization

High-pressure homogenization and microfluidization rely on shear, turbulence, and impact forces for size reduction. Both techniques are more readily adapted for continuous production than probe sonication, but pressure parameters, pass numbers, lipid concentrations, and temperatures must be strictly controlled. Five microfluidization passes at 25,000 psi yielded black rosehip liposomes of ~93.9 nm, whereas subsequent chitosan coating altered surface charge and increased total phenolic-based encapsulation values [23].

Freeze-Thaw and Lyophilization

Repeated freeze-thawing can cause reorganization of the membranes leading to mixing of the internal compartments, but there is a risk of size increase and leakage. When appropriate cryoprotectants and rehydration protocols are developed, lyophilization can be used to support a long-term shelf life Lyophilization increased size and PDI for proliposomes of *Thymus serpyllum* extract containing ergosterol. Formulations with more sterol were structurally more resilient [11]. Consequently, dry stabilization must be evaluated post-rehydration rather than solely on powder appearance.

Tabel 2. Impact of post-processing techniques on liposome properties

Post-processing technique	**Mechanism**	**Expected effects**	**Main risks**	**Critical parameters**	**Reference(s)**
Probe sonication	Cavitation and high-energy shear forces	Reduces particle size and PDI	Heating, lipid oxidation, degradation of herbal constituents, and cargo leakage	Amplitude, processing time, pulse mode, and temperature	Arabi et al., 2017; Akhlaghi et al., 2022
Extrusion	Repeated deformation of vesicles through membranes with defined pore sizes	Produces a more controlled particle size and narrower size distribution	Cargo loss, membrane clogging, and leakage after repeated cycles	Membrane pore size, number of passes, temperature, and lipid concentration	Kurt and Aslan, 2025; Chonsut et al., 2026
Microfluidization	High-pressure shear, turbulence, and particle collision	Produces small and relatively homogeneous vesicles	Temperature increase, equipment costs, and possible cargo degradation	Pressure, number of passes, flow rate, and temperature	Dağ and Oztop, 2017; Özgüven et al., 2021
Freeze–thaw cycling	Repeated freezing and thawing causing bilayer restructuring and fusion	Alters lamellarity and internal aqueous volume and may improve encapsulation	Aggregation, increased particle size, or cargo leakage	Number of cycles and freezing and thawing rates	Nsairat et al., 2022
Lyophilization	Removal of water through freezing and sublimation	May improve long-term storage stability	Increased particle size and PDI after rehydration	Cryoprotectant type and concentration, freezing rate, and rehydration conditions	Jovanović et al., 2025b

Influence of Preparation on Physicochemical Characteristics

Particle Size and Polydispersity Index

The most optimized formulations described in the literature are in the range of 80–400 nm, although some systems without size reduction or with particular compositions are larger than 400 nm. To compare particle sizes directly, processing stages must be taken into consideration. Thin-film hydration leads to the formation of large vesicles which are then converted to smaller vesicles by sonication or extrusion. Therefore, reporting the primary method alone without post-processing may lead to wrong conclusions.

PDI represents the width of the size distribution. Values below 0.30 are generally representative of a relatively uniform population for nanocarrier systems, while higher values are indicative of heterogeneity that may adversely affect stability and reproducibility. White tea (0.06), *Platanus orientalis* (0.11), *Centella asiatica* (0.18) and flavonoid-rich fractions of *Albizia lebbeck* (0.198) liposomes have been reported for low values [4,16,27,28].

Zeta Potential and Colloidal Stability

Most liposomes of herbal extracts have negative zeta potentials, usually from -20 to -60 mV. This charge is due to anionic lipids, constituents of the extract, or surface phosphate groups. Higher absolute values of zeta potential increase electrostatic repulsion and decrease aggregation, but zeta potential alone does not determine stability [2]. Also contributing are steric stabilization by PEG or polymers, medium viscosity, pH, ionic strength and size. Green tea formulations prepared using Mozafari method were stable with zeta potential of -59.7 mV in spite of size of 419 nm [6]. Low surface charge formulations, in contrast, can be stable in the presence of steric protection or under appropriate storage conditions.

Encapsulation Efficiency

The most heterogeneous parameter reported is the encapsulation efficiency. Reported values range from ~17% to almost 100%, with most of the optimized formulations in the range of 60% to 90%. This variation cannot simply be explained by the method of vesicle formation. Hydrophilic extracts require sufficient volume of aqueous phase; lipophilic components depend on bilayer capacity and fluidity. Additional modulation of the results is achieved by extract-to-lipid ratios, cholesterol levels, degree of fractionation, pH, temperature and post-processing energy.

Analytical methodology must also be taken into account. The assays based on total polyphenols can give higher values than the individual marker compound measurements, because each component has a different partition coefficient. The total efficiency of *O. stamineus* extract was 66.2%, and individual marker compound efficiencies were different according to polarity [7]. Differences between the fluorescence and HPLC methods were also reported for liposomal curcuminoids [8]. Thus, comparisons of EE across studies should specify the target analyte and free-fraction separation methods

In Vitro Release

Many of the formulations show a biphasic release. This is characterized by initial burst release of surface localized extract components followed by sustained release through bilayer diffusion or vesicle reorganization . The Higuchi and Korsmeyer-Peppas models are often used to describe the sustained phase [29]. This pattern has been reported for green tea , *Piper longum* , *Glycyrrhiza glabra* , *Albizia*

lebbeck and *Bacopa monnieri* [13,15,28,30,31]. Slower release is not necessarily better; targets requiring rapid onset may need a burst fraction while sustained topical or oral therapies need longer retention.

Storage Stability

The storage condition of 4 °C is the most consistent condition for keeping the size, PDI, zeta potential and encapsulation efficiency constant for 30 to 180 days. Formulations of *Piper longum* were stable for 3 months in cold storage, *Hypericum perforatum* reported stable for up to 180 days at 4 °C and *Platanus orientalis* maintained characteristics for 60 days at ~5 °C [3,13,16,32]. Particle growth or EE reduction is often observed in room temperature storage as in *Albizia lebbeck* and *Berberis vulgaris* [28,33].

The extract components-membrane relation is reciprocal. The bilayer can also prevent phospholipid oxidation as well as protect polyphenol payloads. Black carrot and *Thymus serpyllum* extracts decreased lipid peroxidation in storage or under UV exposure [11,33,34]. However, this protection capacity is limited and excessive amounts of lipids can overcome the antioxidant capacity of the extract.

Table 3. Physicochemical characteristics of selected herbal extract liposomes

Herbal extract	**Preparation method and post-processing**	**Particle size and PDI**	**Zeta potential and EE**	**Release or stability**	**Reference**
Green tea	Thin-film hydration and ultrasonication	99.2 nm; PDI NR	−30 mV; EE 60.09%	Stable for 90 days; biphasic release	Ahmadi et al., 2025
Asparagus racemosus	TF, REV, and PD	197–457 nm; PDI >0.2	−4 to −19 mV; EE 14–68%	Stored at 4°C	Plangsombat et al., 2016
Green tea	Mozafari method	419 nm; PDI 0.28	−59.7 mV; EE 51.34%	Antioxidant activity was maintained for 30 days	Jahanfar et al., 2020
White tea	Thin-film hydration and ultrasonication	82.2 nm; PDI 0.06	−56 mV; EE 76.5%	Improved thermal stability	Ahmadi et al., 2021
Piper longum	Thin-film hydration	104.86 nm; PDI 0.271	−21.8 mV; EE 75.92%	Stable for three months under refrigerated conditions	Himani et al., 2025
Platanus orientalis	Thin-film hydration and sonication	106.6 nm; PDI 0.11	−14.1 mV; EE 72.25%	Stable for 60 days; approximately 75% release within 24 h	Demirel et al., 2025
Ginger	Thin-film hydration and extrusion	94.95 nm; PDI 0.246	Zeta potential NR; EE	Approximately 85% release at pH 6.5	Yavari et al., 2022

			80%		
Carob	Sonication-based preparation	107 nm; PDI 0.27	−13 mV; EE 91–97%	Stability data NR	De Luca et al., 2023
Licorice	Thin-film hydration and sonication	111.4 nm; PDI 0.210	−53.6 mV; EE 75.32%	Approximately 65.35% released within 24 h	Akhlaghi et al., 2022
Hypericum perforatum	Thin-film hydration and extrusion	144.2 nm; PDI 0.247	−24.2 mV; EE 91.83%	Stable for 180 days at 4°C	Kurt and Aslan, 2025
Curcuminoid-rich extract	Thin-film hydration and sonication	110–170 nm; PDI 0.17–0.44	−11 to +53 mV; EE 36–87%	Approximately 10% leakage after four days	Battista et al., 2020
Albizia lebbeck	Thin-film hydration and sonication	138–152 nm; PDI 0.198–0.212	−32.6 to −36.2 mV; EE 67.8–82.5%	Greater stability at 4°C	Lal and Patel, 2026
Centella asiatica	Thin-film hydration and extrusion	120.6 nm; PDI 0.18	−35 to −25 mV; EE 82.5%	Moderate colloidal stability at 4°C	Chonsut et al., 2026
Oregano essential oil	Method not specified in the abstract	Approximately 190 nm; PDI 0.183	−39.8 mV; EE 77.52%	Greater stability at 4°C; biphasic release	Wang et al., 2026
Berberis vulgaris	Aqueous hydration and sonication	166–195 nm; PDI 0.224–0.269	−42.85 to −45.55 mV; EE 40.18–47.19%	Stable for 30 days at 4°C	Homayoonfal et al., 2021

Direct Method-to-Method Comparisons

Comparisons of preparation methods are still scarce but offer the most robust evidence on the effect of process. In the thin-film hydration method, the rosemary oil was not subjected to size reduction, and the resulting particle sizes were in the range of 1054–2748 nm, with PDIs ranging from 0.50 to 0.77. Extrusion generated smaller size of 231-871 nm, while sonication resulted in 92-162 nm with PDIs of 0.12-0.35. However, the encapsulation efficiency in thin film hydration was up to 100%, in sonication was 95-99% and in extrusion was 42-98% [26]. These data suggest that the smallest size does not always give the highest EE.

For a hydrophilic payload, a different relation appears. Thin film hydration of A. racemosus gave multilamellar vesicles of 328–457 nm, whereas reverse phase evaporation and polyol dilution gave oligolamellar vesicles of 197–248 nm. The encapsulation efficiency of 55.71%, 56.21% and 67.68% respectively at an optimal extract to lipid ratio of 1:5 [12]. The benefit seen with hydrophilic saponins is explained by the larger internal aqueous compartment in the oligolamellar vesicles.

The comparisons show that method effects depend on payload localization. For the essential oils used in the bilayer, the maintenance of membrane volume and integrity may be more important than an aqueous core formation. For saponins or hydrophilic polyphenols the decisive factors are the aqueous phase volume and the lamellarity. Therefore, cross-method conclusions should be grouped according to extract polarity and payload localization.

Table 4. Studies directly comparing methods or processing stages

Herbal material	**Methods or processes compared**	**Particle size**	**Encapsulation efficiency**	**Main interpretation**	**Reference**
Asparagus racemosus root extract	TF versus REV versus PD	TF: 328–457 nm; REV: 197–240 nm; PD: 202–248 nm	TF: 55.71%; REV: 56.21%; PD: 67.68% under optimum conditions	PD was the most favorable method for the hydrophilic extract	Plangsombat et al., 2016
Asparagus racemosus root extract	CF versus REV versus PD versus MFD	Varied across formulations	Maximum EE approximately 69.08%	The reported performance order supported PD as the most effective approach in the study	Therdphapiyanak et al., 2013
Rosemary essential oil	Thin-film preparation versus extrusion versus sonication	Thin film: 1054–2748 nm; extrusion: 231–871 nm; sonication: 92–162 nm	Approximately 100%; 42–98%; and 95–99%, respectively	Particle-size reduction did not necessarily increase EE	Arabi et al., 2017
Green tea extract	Microfluidization versus ultrasonication	Detailed values were not available in the abstract	NR	Microfluidization provided better storage stability over one month	Dağ and Oztop, 2017
Phenylethanoside extract	Extrusion versus ultrasonication versus combined processing	Not specified in the review report	Combined treatment increased EE by approximately	The hybrid protocol improved cargo retention	Su et al., 2024

			8% versus extrusion and 18% versus ultrasonication		

Influence of Extract Characteristics and Formulation Composition

Extract Polarity and Fractionation Level

The polarity determines the liposomal compartment in which the payload resides. Sufficient internal compartments are mainly required for saponin- or anthocyanin-rich aqueous extracts, while bilayer fluidity and area are more important for essential oils and lipophilic fractions. Similar methods can be used to change the fractionation level to improve performance. The size of the flavonoid-rich fraction of *Albizia lebbeck* was 138.2 nm, PDI 0.198, zeta potential -36.2 mV, and EE 82.5%; the crude extract was 152.4 nm, PDI 0.212, -32.6 mV, and EE 67.8% [28]. The chemical homogeneity of fractions results in a more uniform interaction with the bilayer.

Extract-to-Lipid Ratio

The extract-to-lipid ratio is the most consistent controllable variable impacting encapsulation. As the amount of lipid increases at a constant extract load, the EE tends to increase as more compartments are available, but the loading capacity per mass of lipid may decrease. Or, by increasing extract without adding lipid, the percentage EE decreases as the bilayer or aqueous core saturates. This pattern has been seen in *A. racemosus, P. longum*, ginseng and Mozafari-based formulations [6,12–14]. EE and loading capacity need to be optimized simultaneously.

Phospholipids and Sterols

Increasing phospholipids increases bilayers and decreases payload density but may increase particle size or decrease relative loading capacity. Cholesterol reduces permeability until a certain threshold, beyond which it may hinder payload interaction if the membrane becomes too rigid. The outcome is dependent on the extract and base lipid. In P. longum, increased lecithin and cholesterol resulted in increased EE and loading capacity decreased due to increased total lipid mass [13]. In *Platanus orientalis* excess cholesterol inhibited sonication-induced size reduction and induced partial aggregation [16].

Solvents and Processing Conditions

Solvents influence component solubility, homogeneity of the lipid extract mixture, rates of film formation and final residues. Matching evaporation rates promote uniform film formation, and differential solvent partitioning can lead to extract- or lipid-rich domains. High temperatures can speed the formation of bilayers above the transition temperature, but may harm phenolics or volatile ingredients. In the Mozafari method for green tea, the concentration of phosphatidylcholine and extract showed a higher effect on EE than the variations of processing time and temperature in the tested ranges [6]. Therefore, the process parameters need to be optimized within the relevant formulation spaces, not in isolation.

Biological Performance of Herbal Extract Liposomes

Antioxidant Activity

Antioxidant activity is the most commonly evaluated endpoint. Liposomes are active in storage or under harsh conditions which degrade free extracts. The DPPH radical scavenging activity of green tea was increased from 90.6% in free extract to 93.37% in liposomal formulation. For rosemary it was increased from 84.57% to 92.5% [6,22]. Improvements in cell-free chemical assays are generally small because free extracts have already a high baseline activity.

Chemical assay results may not always correlate with cellular response. Myrtle liposomes showed a DPPH activity of 91%, slightly lower than the free extract (96%), but were more effective in reducing intracellular ROS in fibroblasts to near-basal levels [35]. This difference is probably due to enhanced membrane interaction and intracellular delivery. Therefore, claims of increased antioxidant activity must distinguish between radical scavenging capacity in solution and intracellular biological protection.

Anti-Inflammatory Activity and Wound Healing

The liposomes of *Centella asiatica* decreased TNF-α by 66.8% and IL-1β by 69.5% at 12.5 µg/mL, compared to 52.8% and 56.4% for the free extract. In a rat excision model, the formulation achieved 99.9% wound closure by day 12 [4]. *Platanus orientalis* liposomes also produced ~47% fibroblast wound closure within 24 hours, compared to ~16% for the free extract [16]. These enhancements align with prolonged local exposure and protection of active constituents.

Marrubium vulgare administration of liposomes 100 mg/kg in Marrubium vulgare reduced the writhing induced by acetic acid by 75.9% and the paw edema induced by carrageenan by 72% and was reported to be similar to ibuprofen in the study model [36]. Such in vivo findings support translational value, but comparisons of dose, route, and carrier composition are still needed before clinical conclusions are made.

Anticancer and Cytotoxic Activity

The IC_{50} against B16F10 melanoma cells was reduced from 88.48 µg/mL (free extract) to 59.71 µg/mL, with liposomes of *Piper longum*, without significant toxicity to HEK293 cells under the test conditions [13]. In mice with colorectal cancer, ginger liposomes increased the expression of Bax and IFN-γ and the counts of tumor-infiltrating lymphocytes compared with the free extract. Goji berry formulations based on unsaturated or hydrogenated lipids also decreased cancer cell viability compared to free extracts [2,9].

Anticancer activity is cell-specific, time-dependent, cell death pathway-dependent and carrier-dependent. Small size may stimulate endocytosis, but intracellular dose depends on surface composition, release rate and encapsulated extract concentration. Therefore, the IC_{50} values should not be compared between different extracts without standardizing models and units.

Antimicrobial and Antiviral Activity

Liposomes may improve the dispersion of the hydrophobic component and its contact with the microbial membrane. Encapsulation of thyme essential oil retained >80% payload and increased antifungal activity against *Trichophyton mentagrophytes* [37]. Naderinezhad et al. (2018) reported only curcuminoid extracts in cationic liposomes containing CPC showed activity against MSSA with a MIC of 7 µg/mL and no equivalent effect was observed for free extracts and non-cationic liposomes at the concentrations tested [8]. This indicates surface charge can be more decisive than preparation method.

For antiviral applications, chitosan-stabilized liposomes loaded with *Aesculus hippocastanum* extract increased the HCoV-OC43 inhibition by a factor of up to 16 compared to the carrier-free extract, as

previously reported, with a selectivity index of 58.96 [38]. Analysis showed that the effect was the consequence of intracellular delivery and not merely surface interaction with the virus.

Bioavailability and Pharmacokinetics

Pharmacokinetic evidence suggests more direct benefits than do chemical assays [1]. Silymarin liposomes increased area under the curve (AUC) by about four times as compared to silymarin suspension in a rat model. Nanoliposomes of *Syzygium campanulatum* increased water solubility by ~50%, allowed plasma detection of several marker compounds and extended exposure relative to non-liposomal extract [39]. These findings support the capacity of liposomes to enhance systemic absorption and retention, although correlation with clinical efficacy warrants controlled clinical trials.

Tabel 5. Comparison of biological performance between free extracts and liposomal formulations

Herbal extract	**Biological endpoint**	**Free extract**	**Liposomal formulation**	**Main finding**	**Reference**
Green tea	DPPH radical-scavenging activity	90.6%	93.37%	Moderate improvement in the chemical antioxidant assay	Jahanfar et al., 2020
Rosemary	DPPH radical-scavenging activity	84.57%	92.5%	Increased radical-scavenging activity	Jahanfar et al., 2021
Myrtle berry	DPPH activity and cellular ROS	DPPH: 96%	DPPH: 91%; stronger reduction of cellular ROS	Chemical antioxidant activity and cellular protection did not show the same trend	De Luca et al., 2022
Centella asiatica	TNF-α inhibition	52.8%	66.8%	Enhanced anti-inflammatory activity	Chonsut et al., 2026
Centella asiatica	IL-1β inhibition	56.4%	69.5%	Enhanced anti-inflammatory activity	Chonsut et al., 2026
Platanus orientalis	Fibroblast wound closure	Approximately 16%	Approximately 47%	Approximately threefold	Demirel et al.,

	after 24 h			improvement in wound closure	2025
Piper longum	IC_{50} against B16F10 melanoma cells	88.48 µg/mL	59.71 µg/mL	Increased cytotoxic potency against melanoma cells	Himani et al., 2025
Silymarin	Pharmacokinetic AUC	Reference suspension	Approximately fourfold higher	Increased systemic exposure	Kumar et al., 2019
Aesculus hippocastanum	Antiviral inhibition against HCoV-OC43	Reference free extract	Up to approximately 16-fold higher	Improved intracellular delivery and antiviral activity	Gyurova et al., 2024

CONCLUSION

The most effective method of preparation differs from one herbal extract to another. Thin-film hydration is the most versatile, but the final product characteristics depend heavily on sonication, extrusion or other size reduction techniques. Enhanced encapsulation of hydrophilic extracts can be achieved by larger internal aqueous compartments formed by reverse-phase evaporation and polyol dilution. Ethanol injection is quick and has scalability potential. Proliposome, and Mozafari methods provide other advantages such as storage or less solvent use.

The particle size, PDI, zeta potential and encapsulation efficiency are the results of complex interactions between methods, extract polarities, extract to lipid ratios, phospholipid compositions, sterols, solvents and post-processing energy. Smaller particle size does not necessarily lead to higher EE or better biological activity. Encapsulation within liposomes generally improves stability, release profiles, cellular activities, wound healing, antimicrobial action, and systemic exposure, although the degree of improvement depends on the endpoint and model. Future formulation development should use fit-for-purpose approaches and comparative designs to control compositional variables to definitively assess method, physicochemical, and biological performance relationships.

CONFLICT OF INTERESTS

The authors declare no conflict of interests related to the publication of this paper.

REFERENCES

1. Kumar N, Rai A, Reddy ND, Shenoy R, Mudgal J, Bansal P, et al. Improved in vitro and in vivo hepatoprotective effects of liposomal silymarin in alcohol-induced hepatotoxicity in Wistar rats. Pharmacol Reports. 2019. doi:10.1016/J.PHAREP.2019.03.013
2. Yavari M, Jaafari MR, Mirzavi F, Mosayebi G, Ghazavi A, Ganji A. Anti-tumor effects of PEGylated-nanoliposomes containing ginger extract in colorectal cancer-bearing mice. Iran J Basic Med Sci. 2022;25. doi:10.22038/ijbms.2022.63870.14075
3. Kurt AA, Aslan İ. A Novel Liposomal In-Situ Hydrogel Formulation of Hypericum perforatum L.: In Vitro Characterization and In Vivo Wound Healing Studies. Gels. 2025.

doi:10.3390/gels11030165
4. Chonsut P, Tawanwongsri W, Naphatthalung J, Chokpaisarn J, Charoensup R, Puttarak P, et al. Evaluation of liposome-encapsulated Centella asiatica ethanolic extract for enhanced in vitro and in vivo wound healing. Front Med Technol. 2026. doi:10.3389/fmedt.2026.1740835
5. Ambardekar R, Mahadik K, Paradkar A, Harsulkar A. Enhancement of hepatoprotective efficacy of propolis by fabrication of liposomes, as a platform nano-formulation for multi-component natural medicine. Curr Drug Deliv. 2012. doi:10.2174/156720112802650653
6. Jahanfar S, Ghavami M, Khosravi-Darani K, Jahadi M. Liposomal Green Tea Extract: Optimization and Physicochemical Characterization. J Appl Biotechnol Reports. 2020. doi:10.30491/jabr.2020.231423.1228
7. Aisha A, Majid A, Ismail Z. Preparation and characterization of nano liposomes of Orthosiphon stamineus ethanolic extract in soybean phospholipids. BMC Biotechnol. 2014. doi:10.1186/1472-6750-14-23
8. Battista S, Maggi M, Bellio P, Galantini L, D'Archivio AA, Celenza G, et al. Curcuminoids-loaded liposomes: influence of lipid composition on their physicochemical properties and efficacy as delivery systems. Colloids Surfaces A Physicochem Eng Asp. 2020. doi:10.1016/j.colsurfa.2020.124759
9. Peixoto FB, Defendi R, Sípoli C, Torre LG de la, Bianchi JR de O, Silva GG da, et al. Encapsulation of goji berry extract in nanoliposomes with antitumor effect: A comparative study of unsaturated and hydrogenated lipids via the ethanol injection method. Can J Chem Eng. 2026. doi:10.1002/cjce.70486
10. Sahumena MH, Suryani S, Pratiwi W. Preparasi dan Karakterisasi Sistem Pembawa Liposom dari Ekstrak Etanol Daun Miana (Coleus atropurpureus L. Benth). Indones J Pharm Educ. 2023. doi:10.37311/ijpe.v3i2.20414
11. Jovanović AA, Balanč B, Petrović PM, Čutović N, Marković SB, Djordjević VB, et al. Liposome-Based Encapsulation of Extract from Wild Thyme (Thymus serpyllum L.) Tea Processing Residues for Delivery of Polyphenols. Foods. 2025;14:2626. doi:10.3390/foods14152626
12. Plangsombat N, Rungsardthong K, Kongkaneramit L, Waranuch N, Sarisuta N. Anti-inflammatory activity of liposomes of Asparagus racemosus root extracts prepared by various methods. Exp Ther Med. 2016;12:2790–6. doi:10.3892/etm.2016.3661
13. . H, Mohapatra D, Shreya S, Biswal S, Naik GG, Malang SD, et al. **Development and Characterization of Liposomal Phytoformulation of *Piper longum* L. for Melanoma Therapy**. Pharm Sci. 2025;31:237–50. doi:10.34172/ps.025.42140
14. Zhang D, Tian N, Guo Y, Liu Y, Sun G, Jiang R, et al. Multifunctional Natural Herbal Extracts Liposomal Loaded Hybrid Hydrogel for Skin Improvement and Repair. Nat Prod Commun. 2025. doi:10.1177/1934578X251332366
15. Akhlaghi M, Eftekharivash L, Taebpour M, Afereydoon S, Ebrahimpour M, Mehrizi MZ, et al. Improving the Therapeutic Performance of Glycyrrhiza Glabra Hydroalcoholic Extract Using Liposomal Nano-carriers and Their Characterization. Dis Diagnosis. 2022. doi:10.34172/ddj.2022.09
16. Demirel F, Sağıroğlu AA, Toraman GÖA, Gunaydin-Akyildiz A, Keskin Z, Aydin B, et al. Development and Evaluation of Platanus orientalis L. Extract-Loaded Liposomes for Enhanced Wound Healing. Pharmaceuticals. 2025. doi:10.3390/ph19010032
17. Sebaaly C, Jraij A, Fessi H, Charcosset C, Greige-Gerges H. Preparation and characterization of clove essential oil-loaded liposomes. Food Chem. 2015. doi:10.1016/j.foodchem.2015.01.067
18. Rushmi ZT, Akter N, Mow RJ, Afroz M, Kazi M, Matas M de, et al. The impact of formulation attributes and process parameters on black seed oil loaded liposomes and their performance in animal models of analgesia. Saudi Pharm J. 2016. doi:10.1016/j.jsps.2016.09.011
19. Altıntaş ÖE, Erdoğmuş SF. Development of controlled delivery systems by nanoliposomes of Hypericum perforatum L. extracts. Int J Plant Based Pharm. 2023. doi:10.29228/ijpbp.20

20. Therdphapiyanak N, Jaturanpinyo M, Waranuch N, Kongkaneramit L, Sarisuta N. Development and assessment of tyrosinase inhibitory activity of liposomes of Asparagus racemosus extracts. Asian J Pharm Sci. 2013. doi:10.1016/J.AJPS.2013.07.017
21. Jovanović A, Balanč BD, Volić M, Pećinar I, Živković J, Šavikin K. Rosehip Extract-Loaded Liposomes for Potential Skin Application: Physicochemical Properties of Non- and UV-Irradiated Liposomes. Plants. 2023. doi:10.3390/plants12173063
22. Jahanfar S, Gahavami M, Khosravi-Darani K, Jahadi M, Mozafari MR. Entrapment of rosemary extract by liposomes formulated by Mozafari method: physicochemical characterization and optimization. Heliyon. 2021. doi:10.1016/j.heliyon.2021.e08632
23. Özgüven M, Kasapoğlu KN, Güngör C, Ertürk EN, Özçelik B. İnce Film Hidrasyon ve Mikrofluidizasyon Yöntemleri Kullanılarak Polifenollerin Yüksek Verimle Nanokapsüllere Yüklenmesi. Eur J Sci Technol. 2021. doi:10.31590/ejosat.946290
24. Dağ D, Oztop M. Formation and Characterization of Green Tea Extract Loaded Liposomes. J Food Sci. 2017. doi:10.1111/1750-3841.13615
25. Su L, Zhao D, Huang Q, Zhao X, Chen Q, Rao H, et al. Preparation of pectin-coated and chitosan-coated phenylethanoside liposomes: Studies on characterization, stability, digestion and release behavior. Int J Biol Macromol. 2024;261:129442. doi:10.1016/j.ijbiomac.2024.129442
26. Arabi MH, Hora, Mirzapour A, Ardestani MS ee, Saffari M. Preparation of nanoliposomes containing Rosmarinus offi cinalis L essential oil; A comparative study. Biosci Biotechnol Res Commun. 2017;10:103–8. doi:10.21786/bbrc/10.1/15
27. Ahmadi E, Elhamirad A, Nia NM, Asl MRS, Nia AP. Incorporation of white tea extract in Nano-liposomes: optimization, characterization, and stability. J Sci Food Agric. 2021. doi:10.1002/jsfa.11544
28. Lal P, Patel J. Development, Optimization, and Physicochemical Characterization of Liposomal Formulations Containing Ethanolic Leaf Extract and Flavonoid-Rich Fraction of Albizia lebbeck for Enhanced Hepatoprotective Delivery. Indones J Pharm. 2026. doi:10.22146/ijp.27798
29. Wang Z, Bao Y, Qiu J, Li S, Chen H, Li C. Fabrication, Characterization, and Transcriptomic Analysis of Oregano Essential Oil Liposomes for Enhanced Antibacterial Activity and Sustained Release. Foods. 2026;15:157. doi:10.3390/foods15010157
30. Ahmadi N, Ahranjani PJ, Rashidi L. Encapsulation of Green Tea Extract (<scp>GTE</scp>) in Nanoliposome and Assessment of Its Characterization and In Vitro Release Study of <scp>GTE</scp>. Food Sci & Nutr. 2025;13. doi:10.1002/fsn3.70781
31. Narayanan VA, Sharma A, S R, R. AT, P G, S. P, et al. Bilosomes as a Potential Carrier to Enhance Cognitive Effects of Bacopa monnieri Extract on Oral Administration. J Heal Allied Sci NU. 2022. doi:10.1055/s-0042-1757969
32. Luca M De, Tuberoso C, Pons R, García MT, Morán M, Martelli G, et al. Ceratonia siliqua L. Pod Extract: From Phytochemical Characterization to Liposomal Formulation and Evaluation of Behaviour in Cells. Antioxidants. 2023. doi:10.3390/antiox12061209
33. Homayoonfal M, Mousavi SM, Kiani H, Askari G, Desobry S, Arab-Tehrany E. Encapsulation of Berberis vulgaris Anthocyanins into Nanoliposome Composed of Rapeseed Lecithin: A Comprehensive Study on Physicochemical Characteristics and Biocompatibility. Foods. 2021;10:492. doi:10.3390/foods10030492
34. Guldiken B, Gibis M, Boyacioğlu D, Çapanoğlu E, Weiss J. Physical and chemical stability of anthocyanin-rich black carrot extract-loaded liposomes during storage. Food Res Int. 2018. doi:10.1016/j.foodres.2018.03.071
35. Luca M De, Lucchesi D, Tuberoso C, Fernàndez-Busquets X, Vassallo A, Martelli G, et al. Liposomal Formulations to Improve Antioxidant Power of Myrtle Berry Extract for Potential Skin Application. Pharmaceutics. 2022. doi:10.3390/pharmaceutics14050910
36. Lefrioui Y, Sepe F, Conte R, Calarco A, Ouedrhiri W, Chebaibi M, et al. Polyphenol-Loaded Liposomal Nanocarriers from Marrubium vulgare: A Promising Nutraceutical Delivery System

with Enhanced Bioactivity and Safety. Molecules. 2026. doi:10.3390/molecules31152660
37. Naderinezhad S, Babasafari S, Haghiralsadat F. Experimental evaluation of biocompatible pegylated phospholipid formulation containing Thyme essential oils and evaluation of anti-fungal activity of nanoscale system. J Gorgan Univ Med Sci. 2018.
38. Gyurova A, Milkova V, Iliev I, Lazarova-Zdravkova N, Rashev V, Simeonova L, et al. Anti-Coronavirus Activity of Chitosan-Stabilized Liposomal Nanocarriers Loaded with Natural Extracts from Bulgarian Flora. Life. 2024. doi:10.3390/life14091180
39. Memon AH, Memon O, Rahman AU, Khan M, Lashari HM, Suheryani I, et al. Stability, in vitro release, and pharmacokinetic evaluation of standardized crude extracts, isolated compounds, and their nano-liposomes derived from Syzygium campanulatum Korth using Sprague-Dawley rats. Drug Dev Ind Pharm. 2026. doi:10.1080/03639045.2026.2631666