A Comprehensive Review: Phytosomes as Innovative Delivery System

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Abstract: - Phytochemicals found in medicinal plants have become a great treatment choice for a wide range of illnesses these days. Poor selectivity and bioavailability, however, may restrict their clinical use. Consequently, bioavailability is regarded as a significant obstacle to enhancing bio-efficacy in the transportation of phytochemicals found in food. Several techniques have been put forth to create efficient carrier systems that increase phytochemical bioavailability. One of these, nano-vesicles, has been proposed as a viable option for delivering phytochemicals that are insoluble. The scientific literature has endorsed and used bilayer vesicles extensively because of their versatility and ease of manufacturing. The current review highlights the major therapeutic applications of phytosomes till date. The review discusses about the latest and novel drug delivery system viz. "Phytosome technology" whereby major emphasis has been provided on the different therapeutic applications of phytosomes and its crucial role in managing the conventional complications that are encountered for the delivery of phytoconstituents. Applications of phytosomes such as enhancing bioavailability, anti-cancer and anti-oxidant agents, transdermal delivery, wound healing capacity etc. have been discussed. The comparison between the therapeutic

activities of free drug and its phytosomes indicates that the complex provides various advantages over conventional treatments.

Keywords: - Phytosome · Nano drug delivery · Phosphatidylcholine · Natural products

Abbreviations:

[NDDS] Novel drug delivery system

[PC] Phosphatidylcholine

[PS] Phosphatidylserine

[VDDS] Vesicular drug delivery system

1. Introduction

Natural products are the treasurable blessings given by nature to the human being. Natural products have been used for their therapeutic purposes since ages. Hundreds of phytoconstituents present in single natural product work together and impart therapeutic benefits. Natural products are not only being the oldest mode of treatment but the safest, effective and easily available mode of treatment also **[1].** Although having so many insignificant properties, natural products rely the most on basis of knowledge and clinical experience of the practitioners and formulators. Natural products are usually obtained by different processes such as extraction, expression distillation, fractionation, purification etc. from whole plant or its parts. However, the particular part or the method to be implemented for specific treatment entirely depends on the manufacturer. Allopathic medicines are currently more popular than traditional ones, especially in developed countries as there are no any standardized natural products having definite therapeutic value. However, most developing countries are continuously using the natural medicines, probably because of high cost of synthetic drugs [**2,3].** This ever-increasing dependency over the modern drugs and failure in treatment of various diseases motivates the scientists to find a better alternative. Low solubility is among the main constraints for using the herbal products for therapeutic purposes. On the other hand, despite the potential efficacy of herbal medicines, they are usually not preferred because of lack of standardization and poor apparent quality. While dealing with herbal extracts, several components are predisposed to degradation in the acidic gastric medium whereas majority of them get broken down inside liver ahead of entering into systemic circulation. Furthermore, natural extracts are oftenly hygroscopic, less compressible and show poor powder flowability. Since plant extracts have plentiful therapeutic significance, now the efforts are being

focused on developing their newer carrier systems to tackle the limitations of conventional dosage forms with reduced efficacy of herbal medicines **[4,5].**

The term "Phytosomes" comprises of two words i.e. 'Phyto' meaning Plant and 'Some' meaning cell-like. They are micelles obtained by the complexation of phospholipids with water and this type of complexation is exaggerated by the adding polyphenolic plant extract. The phytosome technique was first established by Indena, an Italian Company. Bombardelli and Spelta in 1991 developed a novel drug delivery system called Phytosomes **[6].** This technique is categorized under VDDS which encompasses aquasomes, ethosomes, liposomes, niosomes, and phytosomes. VDDS are systems consisting of a hydrophilic core and outer lipid bilayer shell **[7].** The fatty bilayer of phytosomes assists 'contact-facilitated drug delivery' which comprises of lipid-lipid interaction between the cell membrane and carrier, causing diffusion of phytoactive constituents inside the cell **[8].** Phytosomes, also known as herbosomes, is a novel phytolipid formulation technology attempting to break through the barriers put forth by the conventional drug delivery systems with respect to bioavailability and stability of plant-derived drugs. It is vesicular drug delivery system where phytoactive constituents of natural product are surrounded by lipid, consequently forming a formulation with better absorption than the conventional ones **[9].** It is a biocompatible and biodegradable delivery system formed via complexation in a stoichiometric ratio of a phytoactive chemical, or a mixture of phytochemicals, with a phospholipid, mainly phosphatidylcholine or phosphatidylserine in an aprotic solvent **[10].** The spectral values disclose++++++++++ the phospholipid-phytoconstituent interactions due to hydrogen bond formation between the hydrophilic part and the polar parts of the phytoactive constituent **[11].** The average size of a phytosome differs from 50 nanometres to some hundred micrometres **[12].** Phytosome preserve the key components of natural product extract from damage by digestive chemicals and bacteria, as a result of which, improved absorption and bioavailability lead to elevated biological and pharmacokinetic parameters as compared to conventional herbal extracts **[13]**.

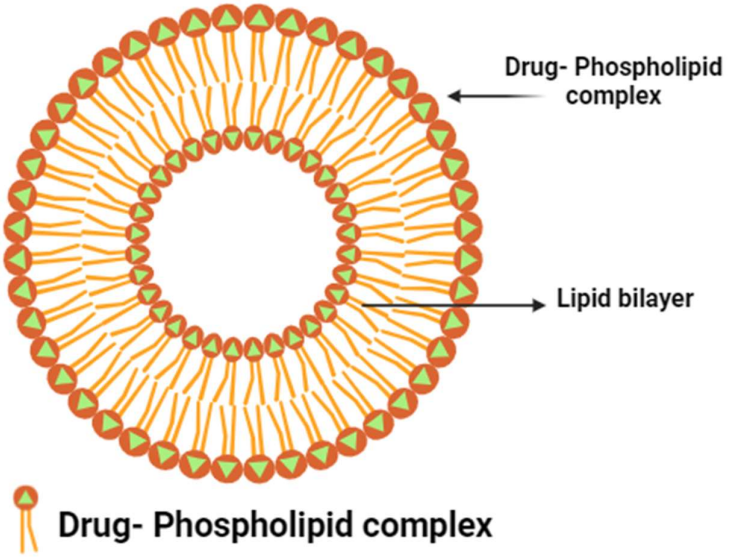

Fig. 1.1 General structure of phytosomes depicting the drug-phospholipid complex in which the choline head of phosphatidylcholine (PC) binds to the phytoactive constituent and the tail encapsulates the polar portion of the complex to provide a hydrophobic surface

Indena, an Italy-based pharma company, has manufactured several health care & skin care phytosomal products for use in various health problems. Most of the standardized herbal extracts containing polyphenolics and terpenoid fractions are extensively formulated as phytosomes, some of them are summarized in Table 1.1 **[14].**

Table 1.1 Phytosome-based products developed by various manufacturers along with their biological source and therapeutic significance [Kumar et al. 2019].

Sr. No.	Brand name	Botanical origin & plant part used	Therapeutic significance	References
1)	18 Beta-glycyrrhetinic acid phytosome	*Glycyrrhiza glabra* L. root	Lenitive, soothing	**[15]**
2)	Bosexil Phytosome	*Boswellia serrata*	Anti-photo ageing, soothing	**[14]**
3)	Casperome Phytosome	Roxb. Root	Joint health	**[16]**
4)	Virtiva Phytosome	*G. biloba* L. leaf	Cognitive enhancer	**[17]**
5)	Ginseng phytosome	*Panax ginseng* C.A. root	Improves elastic strength of skin	**[18]**
6)	Greenselect phytosome	*Camellia sinensis* L. Kuntze young leaf	Free radical scavenger, weight regulator, whitening agent	**[19]**
7)	Leucoselect phytosome	*Vitis vinifera* L. seed	Antioxidant, cardio-protective, ultra-violet protective	**[20]**

8)	Meriva	*Curcuma longa* L. rhizome	Joint strengthener, soothing	**[21]**
9)	Proanthocyanidin A2 phytosome	*Aesculus hippocastanum* L. bark	Ultra-violet protective promotes skin repair and skin firmness	**[14]**
10)	Quercefit phytosome	Citrus Fruits and onion bulb	Anti-ageing, antioxidant, Sirt1 modulator	**[22]**

1.1 Mechanism of Drug Complexation

Phytosomes are the product obtained by the interaction of a phospholipid (stoichiometric quantity) with the plant extract or phytoactive constituent inside an aprotic solvent **[23].** Phosphatidylcholine is a bi-functional compound in which, the choline part of the molecule binds to the phytoactive constituent whereas the tail (phosphatidyl part comprising of two long lipophilic chains) encapsulates the polar portion of the complex to provide a hydrophobic surface **[24].** Spectral techniques have shown that the phytoactive constituents bind with the choline head through H-bonds thus forming a stable and more bioavailable form of herbal drug delivery **[6].**

1.2 Components of Phytosomes

1.2.1 Phyto-Active Constituents

Herbal drugs are selected on various basis primarily on utility of drug in therapeutics, their nature, availability, estimation method and stability. The nature of phytoactive constituent in the herbal extract is a crucial element in drug selection. Herbal extracts being multi-component mixtures, generally possess multiple ring molecules, owing to large size, are tough to be diffused by simple passive diffusion and depict poor penetrability through the cellular lining of the intestine **[25].** For producing a phytosomal complex, the drug moiety must have active hydrogens like –NH2, –NH, –OH, and –COOH, having the capacity of estabilishing H-bonds between the drug and N-methyl groups of PC **[26].** Previous work has reported that molecules with conjugated systems of pie electrons are proficient in making various types of complexes with phospholipids.

1.2.2 Phospholipid

Phospholipids being amphiphilic and zwitterionic molecules, are considered as a potential component of cell membrane **[27, 28].** They extensively exist in plants and mammals as they are among the necessary constituents of cellular membranes **[29].** Main sources of phospholipids include rape seed, sunflower seed, soya bean, vegetable oils and cotton whereas mammalian tissues include bovine brain and egg yolk **[30].** Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and Phosphatidylserine (PS) are the chief phospholipids largely employed to formulate complexes consisting of an aqueous head group and two lipophilic chains **[31].** However, PC is the most widely employed phospholipid, due to its amphipathic nature which imparts it adequate solubility in water and lipid medium. Furthermore, being a vital component of cell membranes, PC displays strong biocompatibility and little toxicity **[32].** Phosphatidylcholine consists of two aliphatic chains surrounding the choline-phytoactive drug complex as depicted in Fig. 1.2.

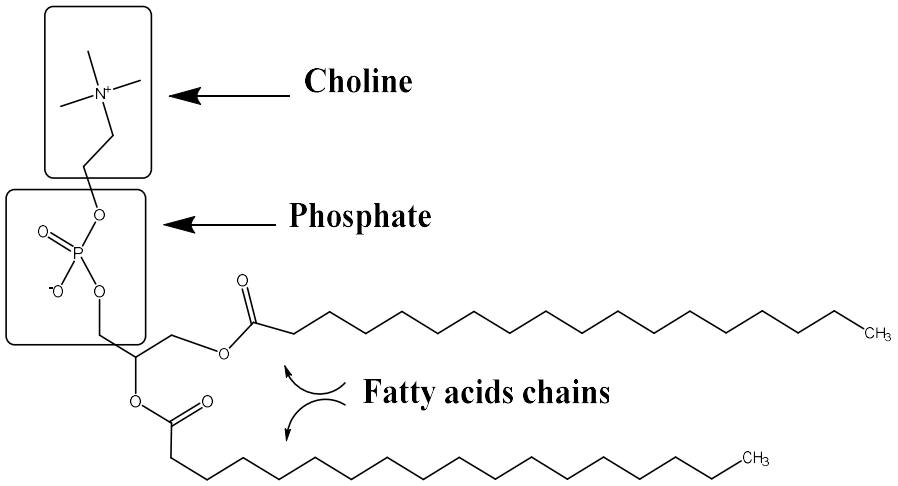

Fig. 1.2 Structure of phosphatidylcholine consisting of a hydrophilic head group and two long lipophilic fatty acid chains

1.2.3 Solvent

During the phospholipid complex formation, the selection of organic solvent plays an important role and relies on the solubilisation parameters of both drug and phospholipids. Previous studies suggested that improved solubility can be achieved by using both protic and aprotic solvents in combination. The majority of the aprotic solvents like chloroform, diethyl ether, dioxane, dichloromethane and n-hexane have been recently substituted with ethyl alcohol being much safer than the earlier solvents **[33].** Since ethyl alcohol leaves less residues and causes

minimal damage, it may be a valuable solvent when the phospholipid complex yield is sufficiently high **[34].**

1.2.4 Stoichiometric Proportion of Active Constituents and Phospholipids

Usually, plant-phospholipid compounds are produced from interaction between natural/synthetic phospholipids and active herbal components in the molar ratios ranging from 0.5 to 2.0. But, according to literature and previous studies, a stoichiometric proportion of 1:1 is considered as the most proficient proportion for formulating complexes of phospholipid **[35, 36].** This is evident from a study where quercetin-phospholipid complexes were formulated by mixing quercetin and Lipoid S100 at a molar proportion of 1:1 **[37].** However, various other proportions of phospholipids and phytoconstituents have also been used.

1.2.5 Properties of Phytosomes

Basically, phytosome is an herbal drug-phospholipid complex formed by reacting stoichiometric amounts of phospholipid and drug in a suitable solvent. Data from spectroscopic studies reveals the key phospholipid-drug interaction owing to the development of hydrogen bonds between the hydrophilic heads of phospholipids (ammonium and phosphate groups) and polar groups of the drug. Phytosomes assume micellar shape due to the presence of water, which makes them look like liposome constructions. Undoubtedly, phytosomes are better forms of natural products which are stable, easily absorbed and thus produce better results than the conventional herbal extracts **[38].** Phytosomes should not to be puzzled with liposomes. Liposomes are formed by mixing a water-soluble substance with phosphatidylcholine without forming any chemical bond, and there may be numerous phosphatidylcholine molecules surrounding the water-soluble compound, whereas in case of phytosomes, the PC and individual phytoconstituent form a 1:1 or a 2:1 complex involving a hydrogen bond formation in presence of suitable solvent **[39].** The comparison between a liposome and a phytosome has been shown in Fig. 7.3 and Table 7.2 **[40, 38].**

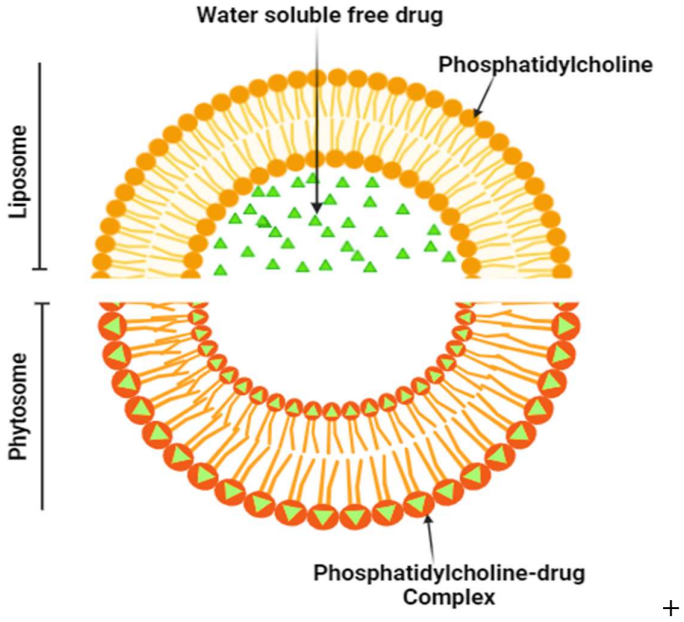

Fig. 1.3 Comparison between phytosome and liposome representing phytosome consisting phospholipid-drug complex and liposome where several PC molecules surround the water-soluble drug [41]

Table 1.2 Comparison of phytosome with liposome based on linkage through bonds, degree of absorption and bioavailability along with molecular arrangements

Characteristics	Phytosome	Liposome
Bond linkage	It is a complex of few molecules united through chemical bonds.	In liposomes, no chemical bond is formed.
Absorption and bioavailability	It affords better absorption and bioavailability	The bioavailability and absorption parameters of liposomes are slighter than phytosomes.
Organisation of molecules	Phospholipid and every phytoconstituent are present in 1:1 or 2:1 proportion.	A large number of phospholipids border the water-soluble phytoconstituents.
Composition	Phospholipids and polyphenolic	phytoconstituents Phospholipids and cholesterol
Flexibility	Rigid	Rigid
Main application	Phyto-delivery	Drug and gene delivery

Administration	Oral, parenteral topical, transdermal	Oral, parenteral topical, transdermal
Key features	High entrapment efficiency along with a depot formation which releases the contents slowly	Biocompatibility, capacity for self-assembly, ability to carry large drug payloads
Limitations	Leaching of the phytoconstituents which reduces the desired drug concentration indicating their unstable nature	Low skin penetration, low stability
Marketed Product	Leucoselect, Greenselect, Panax ginseng, Sabalselect, etc.	Doxil, Abelcet, Visudyne, DepoDur, etc.

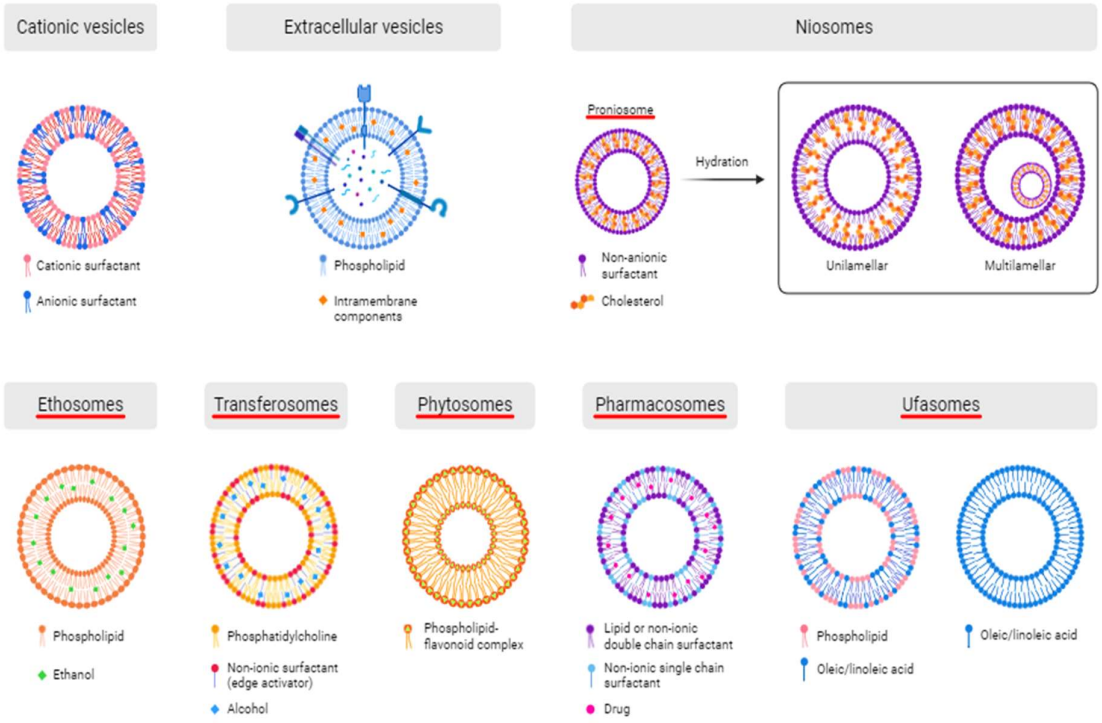

Fig 1.4 Some Lipid-Based Drug Delivery Systems

1.4 Release of Drug from Phytosomes

Phytosomes are preferable while delivering lipophilic herbal drug components. Due to hydrolytic digestion, lipophilic components are likely to develop clusters in the intestine which would obstruct the controlled and constant release of the drug into systemic circulation. This problem is rectified when

lipophilic drug are delivered through phytosomal complexes where the phospholipid develops a monolayer in the digestive track, deters the cluster development, and boosts the dissemination of lipophilic drugs through the small intestine **[42].** The entrapment efficiency of the phytosome reported previousely lies in the range of 86–98%, which could be due to the bond formation between the phytoconstituent and the polar head of the charged phospholipid boosting the association of phytoconstituents with the polar heads. The release of drug from phytosome complex is reported to be timedependent and diffusion controlled. The maximum amount of drug release was estimated nearly 80–85% from the phytosomal complex. However, the rate of drug release from phytosomes is slower than the liposome is due to the linkage of the drug with the phosphatidyl head **[43, 44].** The reason behind firmness of the phytosomal shell can be credited to the timedependent drug release from the complex.

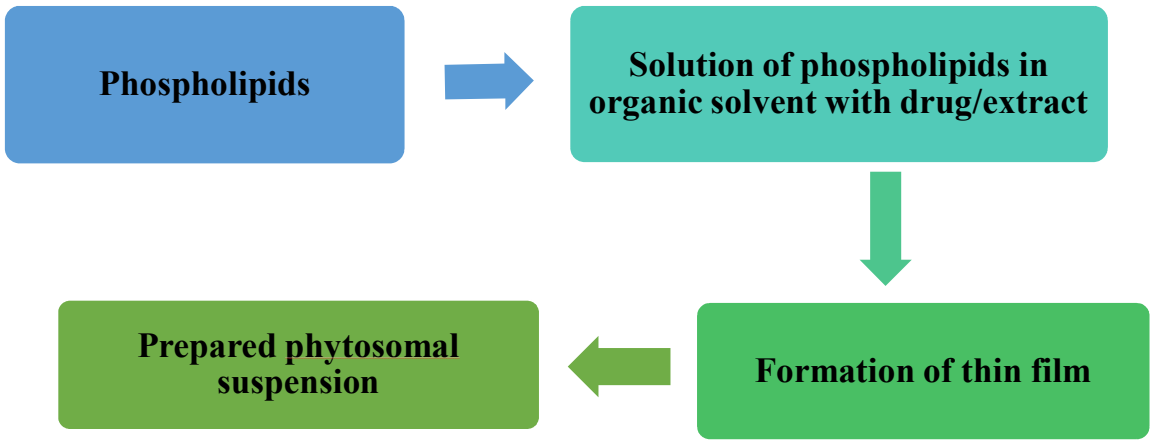

Fig. 1.5 General steps employed in preparation of a phytosome [1].

Further, some patents on Phytosomes are summarized in Table 1.3.

Sr. No.	Patent Number	Patent title	Assignee	Description	References
1	EP1214084	An anti-oxidant preparation based on plant extracts for the treatment of circulation and adiposity problems.	Amsterdam (olanda) - Succursale Di Lugano	Preparation based on plant extracts which has an anti-oxidant effect and is particularly useful in treatment of circulation problems such as phlebitis, varicosevein, arteriosclerosis, haemorrhoid and high blood pressure.	**[46]**

2	EP 0464297	Complexes of neolignane derivatives with phospholipids, the use thereof and pharmaceutical and cosmetic formulations containing them	Ezio Bombardelli, Gianfranco Patri	Complexes of lipophilic extracts from plants of Krameria or Eupomatia genus and of some neolignanes isolated from the same extracts with natural or synthetic phospholipids; said complexes proved to have antiradical, antibacterial and antimycotic activities	**[47]**
3	US 7691422	Oral compositions for the treatment of cellulite	Ezio Bombardelli	oral pharmaceutical and cosmetic compositions for the treatment of cellulite containing Vitis vinifera extracts, Centella asiatica triterpenes and dimeric Ginkgo biloba flavonoids, in the free form or complexed with phospholipids	**[48]**
4	EP 0209038	Complexes of flavanolignanes with phospholipids, preparation thereof and associated pharmaceutical compositions	Bruno Gabetta, Ezio bombardelli	Novel compounds comprising lipophilic complexes of silybin, silidianin, and silicristin with phospholipids, and the preparation of these complexes advantageously be used in the treatment of acute and chronic liver disease of toxic, metabolic or infective origin or of degenerative nature	**[49]**
5	WO 2007/10155	Phospholipid complexes of curcumin having improved bioavailability	Herve,Barriere, Yann Leriche	The phospholipids complexes of curcumin provides higher systemic levels of parent agent than uncomplexed curcumin. The improved bioavailability of phospholipid complex of curcumin increases the potential scope of medical applications for	**[50]**

				curcumin as a chemopreventive agent.	
6	WO/2004/045541	Soluble isoflavone compositions	Anil Khare	Isoflavone compositions exhibiting improved solubility (e.g., light transmittance), taste, colour, and texture characteristics, and methods for making the same	**[51]**
7	EP0283713	Complexes of saponins with phospholipid and pharmaceutical and cosmetic compositions containing them.	Gian Franco Patri	Complexes of saponins with natural or synthetic phospholipid have high lipophilic and improved bioavailability and are suitable for use as active principle in pharmaceutical, dermatologic and cosmetic compositions	**[52]**
8	EP/1844785	Phospholipid complexes of olive fruits or leaves extracts having improved bioavailability	Federico Franceschi	Phospholipids complexes of olive fruits or leaves extracts or their compositions containing it which imparts improved bioavailability	**[53]**
9	EP 0441279	Bilobalide phospholipide complexes, their applications and formulations containing them	Guiseppe Mustich, Ezio Bombardelli	Complexes between natural or synthetic phospholipids and bilobalide, a sesquiterpene extracted from the leaves of Gingko biloba, are disclosed, as well as the preparation thereof and their therapeutic application as antiinflammatory agents and as agents for the treatment of disorders associated with inflammatory or traumatic neuritic processes. These new compounds, which exhibit a different bioavailability compared with free bilobalide, are suitable for incorporation into pharmaceutical formulations for	**[54]**

				systemic and topical administration	
10	EP1813280	Compositions comprising Ginkgo biloba derivatives for the treatment of asthmatic and allergic conditions	Francesco Di Pierro	Compositions containing fractions deriving from Ginkgo biloba, useful for the treatment of asthmatic and allergic conditions	**[55]**
11	EP 0275005	Complex compounds of bioflavonoids with phospholipids, their preparation and use, and pharmaceutical and cosmetic compositions containing them	Ezio Bombardelli	Complex compounds of flavonoids with phospholipids, characterized by high lipophilia and improved bio-availability and therapeutic properties as compared with free, not complexed flavonoids.	**[56]**
12	US/2007/ 0015698	Treatment of skin, and wound repair, with thymosin β-4	Robert Llewellyn Clancy, Gerald Pang	The formulation developed containing Thymosin β4 for wound healing	**[57]**
13	US6297218	Phospholipid complexes prepared from extracts of Vitis vinifera as anti-atherosclerotic agents	Paolo Morazzoni	Vitis vinifera extract phospholipid complexes for the prevention and treatment of atherosclerosis.	**[58]**
14	EP1640041	Cosmetic and dermatological composition for the treatment of aging or photodamaged skin	Thomas Doring	The topical cosmetic or dermatological preparation containing at least one collagen synthesis-stimulating agent for anti-wrinkle treatment	**[59]**
15	WO2009/101551	Phospholipid complex of curcumin having improved bioavailability	Leendert Van Der Tempel	Phospholipid complexes of curcumin provide a higher systemic level of parent agent than uncomplexed curcumin.	**[60]**

2. Methods of Preparation

There are different techniques or methods employed in the preparation of phytosomes.

2.1 Solvent Evaporation Method

Here, both the phytoactive constituents and phospholipid are mixed in a container having an organic solvent and is usually kept at 40 °C for 1 h to achieve maximum entrapment of the drug in the phytosomes so designed followed by removing the organic solvent using rotary evaporator. Then, sieve (100 mesh size) is used for separating thin film phytosomes, which are stored in desiccators for overnight **[61].**

2.2 Salting out or Anti-solvent Precipitation Method

In this method, the designated phytoactive constituent and phospholipid both are kept inside a flask containing some suitable organic solvent and then, the mixture is refluxed at a defined temperature for a specified duration. Later, the solution gets concentrated followed by adding anti-solvent like n-hexane **[62].** Phospholipid complex is then formed as precipitates which are further subjected to vacuum filtration and preserved in airtight amber-coloured glass containers.

2.3 Mechanical Dispersion Method

In this process, the phospholipids dissolved in a suitable solvent are kept in vicinity with the aqueous phase comprising the herbal extract. Firstly, phospholipid dissolved in organic solvent, is slowly introduced to an aqueous solution containing the phytoactive constituents to be complexed with. Consecutive removal of the organic solvent under vacuum results in phyto-phospholipid complex development. Recent approaches for formulating such complex include supercritical fluids, further encompassing compressed antisolvent process, gas antisolvent method and supercritical antisolvent methods **[63].**

2.4 Lyophilization Technique

In this method, both phospholipid and phytoactive constituent are dissolved in respective solvents and further, the solution containing phytoconstituent is added to a solution having phospholipid followed by stirring till complex formation occurs which is separated by lyophilization **[64].**

3. Characterization of Phytosomes

3.1 Spectroscopic Evaluation

Phytoactive constituents-phospholipid complexation and their molecular relationships are investigated using different spectral techniques like 1H-NMR, 13C-NMR, and IR **[65].**

1H-NMR

Bombardelli et al. studied and compared the NMR spectra of (+) cathechin, a flavanoid and its stoichiometric complex with disteroyl-phosphatidylcholine. In non-polar solvent, a marked change was observed in proton nuclear magnetic resonance signal coming from the atoms involved the complex formation, without adding signals of individual molecules. The signals observed from flavonoids are broadened depicting that the protons cannot show along with the broadening of all the signals in phospholipids, while the singlet peak analogous to N-methyl of choline undergoes an uplift shift. The signal revealed that disteroyl-phosphatidylcholine conceals the signal from polyphenol which attributes in complex formation **[66].**

13C NMR

13C-NMR spectral data of the above-mentioned cathechin-disteroylphosphatidylcholine complex, especially when recorded in deuterated benzene at room temp. Indicates that all the flavonoid carbons are hidden. Some signals representing glycerol and choline portion of the lipid are shifted and broadened, while most of the resonance of the fatty acid chain holds their original sharp line shape. Chemical shifts of 13C nuclei in various rings of flavonoids and the choline of phosphatidylcholine helped in understanding their mechanism of interaction **[66].**

FTIR

The recorded IR spectral values are interpreted for different functional groups at their respective wave number **[67].** It confirms the phytosomal complex formation by comparing the IR values of individual constituents and their physical mixture with those of the complex. This technique is also used to determine and ensure the permanency of prepared complexes. The permanency is further ascertained by comparing the spectrum of phytosomal formulation (solid form) with that of micro-dispersion (in water) after lyophilisation at different time intervals.

3.2 Visualization

The Phytosomal formulations can be visualized either by TEM or SEM techniques. In SEM, the sample (dried) is placed on an electron microscope brass stub, coated with gold in an ion sputter whereas in TEM, transmitted electrons are used to divulge the surface morphology with interior structure and crystallographic nature of the sample along with it is used to depict the size of phytosomal vesicles **[68].**

3.3 Morphological Evaluation

Morphological parameters like particle size of phytosome etc. are measured by dynamic light scattering particle size analyser **[69].**

3.4 Entrapment Efficiency

Entrapment efficiency is can be estimated by centrifugation method **[70].** Phytosomal formulations kept inside a centrifuge tube are centrifuged at 14,000 rpm for 30 min. The collected supernatant (1 mL) is diluted with a phosphate-buffered solution having pH 7.4 or distilled water. Then, the concentration of entrapped phytoactive constituent (drug) is determined by a UV-visible spectrophotometer at a wavelength at which the maximum peak (λ max) is obtained for that constituent.

% Entrapment = Total drug Diffused drug/ Total drug * 100

3.5 Vesicle Stability

The average size and structure of vesicles contributing towards vesicle stability are determined by Dynamic Light Scattering and Transmission Electron Microscopy, respectively **[71].**

3.6 Crystallinity and Polymorphism

X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) are widely used techniques for determining crystallinity and polymorphism. In DSC, interactions in phyto-phospholipid complex are characteristically identified with the appearance of new peaks, abolition of endothermic peaks, change in peak intensity, shape and its onset, relative peak area, peak temperature and melting point, or enthalpy etc. In XRD, phyto-phospholipid complexes are analysed either by reduction or complete absence of the intensity of large diffraction peaks corresponding to its crystalline drug **[72].**

3.7 In-vitro Drug Release

Determination of the permeation rate can be achieved by in-vitro drug release study. For calculating the drug release, phytosomal suspension is placed in a Franz diffusion chamber where the samples are collected at different time intervals. Then, from the amount of drug entrapped at 0 times as the initial amount, the quantity of drug unconfined is measured indirectly **[73].**

4. Recent Research Work on Phytosomes

i. Soy phytosome based thermosgel as topical anti-obesity formulation:

Nano lipovesicles thermogel of Soybean, Glycine max (L.) Merrill, was prepared by solvent evaporation, co-solvency and salting out techniques and its optimized formulation was then selected for further analysis by FTIR and Zeta analyser. Prepared phytosomes were then incorporated into a thermogel formulation which showed local anti-obesity effect in male albino rats **[74].**

ii. Development of antidiabetic phytosomes:

Antidiabetic phytosomes of methanolic fruit extract of three plants using a three-factor, three-level Box-Behnken design (17 batches) were optimized and characterized. TEM data revealed their improved steadiness and a spherical shape. Optimized formulation gave maxi mum yield and showed the highest entrapment efficiency. Their antidiabetic effect was equivalent to the standard drug (metformin) at a low dose level **[75].**

iii. Nano-phytosomes of Silibinin and Glycyrrhizic acid:

Ochi and colleagues developed nano-phytosoms of silibinin and glycyrrhizic acid to target liver HepG2 cell lines. Reports showed that such encapsulation of both drugs not only improved the therapeutic potential and permanency of silibinin but also synergistically increased the therapeutic effect. **[76]**

iv. L-carnosine phytosomes for ocular delivery:

A novel phytosomal formulation was prepared for ocular delivery of L-carnosine by blending hyaluronic acid hydrogel and phospholipid by solvent evaporation method and the results witnessed improvement in spreading capacity, sustained infusion, rheological and tolerability features for successful delivery of the drug **[77].**

v. Sinigrin phytosomes for in-vitro skin permeation:

Mazumder et al. developed phytosome complex of sinigrin with in-vitro skin permeation ability and showed the desired release of the drug from the complex and also proposed the probability of exploiting this formulation for significant delivery of this drug to the skin **[78].**

vi. Phyto-liposomes of Silybin-phospholipid complex:

Silybin phospholipid complex by reverse-phase evaporation technique targeted human hepatoma cells and expressed three hundred times more potent biological effect **[79].**

vii. Development of self-nano emulsifying drug delivery system (SNEDDS) of Ellagic acid:

prepared a phytosome complex of ellagic acid-phospholipid by the antisolvent process, followed by developing SNEDDS. Study outcomes reported that this technique can be served as a prominent method to develop formulations of phytoconstituents with limited bioavailability **[80].**

viii. Phytosomal formulation of Polyphenolic extracts of Persimmon (*Diospyros kaki* L.) fruit:

Phytosomes (less than 300 nm) of polyphenolic-rich fruit extract were successfully prepared encapsulating 97.4% of total phenolics, and exhibited higher antioxidant activity **[81].**

Conclusion

Phytosome drug delivery systems have shown excellent results in improving the bioactivity and pharmacological properties of natural product from plants. Phytosome drug delivery system is able to improve solubility and penetration of active compounds through biological membranes allowing the maximum bioavailability. In addition, the capability to mediate controlled release systems, targeted delivery systems, and being able to increase the stability of active compounds make it as the first choice to increase the effectiveness and become a promising technique for pharmaceutical products. Phospholipids show affinity for active constituents through hydrogen bond interactions. Bioavailability can be significantly improved with the help of phospholipids compared with chemically equivalent non-complexed forms. The potential of phytophospholipid complexes, with the effort of clinicians and other researchers, has a bright future for applications in the pharmaceutical field.

References

1) Kumar D, Vats N, Saroha K, Rana AC. Phytosomes as emerging nanotechnology for herbal drug delivery. Sustainable Agriculture Reviews 43: Pharmaceutical Technology for Natural Products Delivery Vol. 1 Fundamentals and Applications. 2020:217-37.
2) Handa SS, Khanuja SPS, Longo KG, Rakesh DD (eds) (2008) Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology, Italy
3) Bonifacio B, Bento P, Dos M, Ramos S, Bauab T, Chorilli M, Negri S (2014) Nanotechnologybased drug delivery systems and herbal medicines: a review. Int J Nanomedicine 9(1):1–15.
4) Aqil F, Munagala R, Jeyabalan J, Vadhanam MV (2013) Bioavailability of phytochemicals and its enhancement by drug-delivery systems. Cancer Lett 334(1):133–141.
5) Byeon JC, Ahn JB, Jang WS, Lee SE, Choi JS, Park JS (2019) Recent formulation approaches to oral delivery of herbal medicines. J Pharm Investig 49(1):17–26
6) Bombardelli E (1991) Phytosome: new cosmetic delivery system. Boll Chim Farm 130(1):431–438
7) Azeez N, Sivapriya V, Sudarshana D (2017) Phytosomes: emergent promising nano vesicular drug delivery system for targeted tumor therapy. Int Res J Pharm 8(34):1–4.
8) Bhupinder K, Reena G, Sachin K, Monica G, Saranjit S (2018) Prodrugs, phospholipids and vesicular delivery – an effective triumvirate of pharmacosomes. Adv Colloid Interf Sci 5(2):233–235.
9) Pawar HA, Bhangale BD (2015) Phytosome as a novel biomedicine: a microencapsulated drug delivery system. J Bioanal Biomed 7(1):006–012.
10) Karatas A, Turhan F (2015) Phyto-phospholipid complexes as drug delivery system for herbal extracts. Turkish J Pharm Sci 12(1):93–102
11) Tripathy S, Patel D, Baro L, Nair S (2013) A review on phytosomes, their characterization, advancement and potential for transdermal application. J Drug Deliv Ther 3:147–152.
12) Patel A, Tanwar Y, Rakesh S, Patel P (2013) Phytosome: Phytolipid drug delivery system for improving bioavailability of herbal drug. J Pharm Sci Biosci Res 3:51–57.

13) Lu M, Qiu Q, Luo X et al (2019) Phyto-phospholipid complexes (phytosomes): a novel strategy to improve the bioavailability of active constituents. Asian J Pharm Sci 14(3):265–269.

14) Kumar S, Baldi A, Sharma DK (2019) Phytosomes: a modernistic approach for novel herbal drug delivery-enhancing bioavailability and revealing endless frontier of phytopharmaceuticals. J Dev Drugs 8(1):1–8.

15) Bombardelli E, Curri SB, Della Loggia R, et al. AntiInflammatory Activity of 18-ß-Glycyrrhetinic Acid in Phytosome Form. Fitoterapia. 1989;60:29–37.

16) Riva A, Allegrini P, Franceschi F, et al. A novel boswellic acids delivery form (casperome (R)) in the management of musculoskeletal disorders: a review. Eur Rev Med Pharmacol Sci. 2017;21 (22):5258–5263

17) Di Pierro F, Togni S, Franceschi F, Eghenhofner R, Giacomelli L. Effects of standardized Ginkgo biloba extract complexed with phosphatidylserine (Virtiva®) on physiological response to prolonged, intense physical activity. Minerva Ortop Traumatol. 2016;67(3):119–123.

18) Kiefer DS, Pantuso T. Panax ginseng. Am Fam Physician. 2003;68(8):1539–1542

19) Di Pierro F, Menghi AB, Barreca A, Lucarelli M, Calandrelli A. GreenSelect (R) phytosome as an adjunct to a low-calorie diet for treatment of obesity: a clinical trial. Altern Med Rev. 2009;14 (2):154–160

20) Magrone T, Pugliese V, Fontana S, et al. Human use of leucoselect (R) phytosome (R) with special reference to inflammatory-allergic pathologies in frail elderly patients. Curr Pharm Des. 2014;20 (6):1011–1019.

21) Drobnic F, Riera J, Appendino G, et al. Reduction of delayed onset muscle soreness by a novel curcumin delivery system (Meriva®): a randomised, placebo-controlled trial. J Int Soc Sports Nutr. 2014;11(1).

22) Cesarone MR, Belcaro G, Hu S, et al. Supplementary prevention and management of asthma with quercetin phytosome: a pilot registry. Minerva Med. 2019;110(6):524–529

23) Patel J, Patel R, Khambholja K, Patel N (2009) An overview of phytosomes as an advanced herbal drug delivery system. Asian J Pharm Sci 4(6):363–371.

24) Amit P, Tanwar Y, Rakesh S, Poojan P (2013) Phytosome: Phytolipid drug delivery system for improving bioavailability of herbal drug. J Pharm Biol Sci 3(1):51–57.

25) Sarika D, Khar R, Chakraborthy G, Saurabh M (2016) Phytosomes: a brief overview. J Pharm Res 15(2):56–62.

26) Semalty A, Semalty M, Rawat B, Singh D, Rawat M (2009) Pharmacosomes: the lipidbased novel drug delivery system. Expert Opin Drug Deliv 6(6):599–612.
27) Olsson N, Salem N (1997) Molecular species analysis of phospholipids. J Chromatogr B Biomed Sci Appl 692(2):245–256.
28) Singh RP, Gangadharappa H, Mruthunjaya K (2017) Phospholipids: unique carriers for drug delivery systems. J Drug Deliv Sci Technol 39:166–179.
29) Seimiya T, Ohki S (1973) Ionic structure of phospholipid membranes, and binding of calcium ions. Biochim Biophys Acta Biomembr 298(3):546–561.
30) Chaurio R, Janko C, Munoz L, Frey B, Herrmann M, Gaipl U (2009) Phospholipids: key players in apoptosis and immune regulation. Molecules 14(12):4892–4914.
31) Suriyakala P, Babu N, Rajan D, Prabakaran L (2014) Phospholipids as versatile polymer in drug delivery systems. Int J Pharm Sci 6(1):8–11.
32) Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X (2015) A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci 10(2):81–98.
33) Shakeri A, Sahebkar A (2016) Phytosome: a fatty solution for efficient formulation of phytopharmaceuticals. Recent Pat Drug Deliv Formul 10(1):7–10.
34) Patel J, Patel R, Khambholja K, Patel N (2009) An overview of phytosomes as an advanced herbal drug delivery system. Asian J Pharm Sci 4(6):363–371
35) Bombardelli E (1994) Phytosome in functional cosmetics. Fitoterapia 65(5):387–401
36) Bombardelli E, Spelta M, Loggia D, Sosa S, Tubaro A (1991) Aging skin: protective effect of Silymarin-phytosome. Fitoterapia 62(2):115–122
37) Zhang K, Zhang M, Liu Z (2016) Development of quercetin-phospholipid complex to improve the bioavailability and protection effects against carbon tetrachloride-induced hepatotoxicity in SD rats. Fitoterapia 113:102–109.
38) Kumar A, Kumar B, Singh S et al (2017) A review on phytosomes: novel approach for herbal phytochemicals. Asian J Pharm Clin Res 10(10):41–47.
39) Suryawanshi JAS (2011) Phytosome: an emerging trend in herbal drug treatment. J Med Genet Genomics 3(6):109–114
40) Gandhi A, Dutta A, Pal A, Bakshi P (2012) Recent trends of phytosomes for delivering herbal extract with improved bioavailability. J Pharmacogn Phytochem 1(4):6–14
41) Akki R, Sri KN, Govardhani KL, Ramya MG (2019) Phytosomes: a novel drug delivery for herbal extracts. Res J Life Sci Bioinforma Pharm Chem Sci 5(2):1069–1082.

42) Hamed M, Abolfazl S, Bahman R, Amin J, Zarrin B, Amirhossein S (2017) Phytosomal curcumin: a review of pharmacokinetic, experimental and clinical studies. Biomed Pharmacother 85:102–112.

43) Amit P, Tanwar Y, Rakesh S, Poojan P (2013) Phytosome: Phytolipid drug delivery system for improving bioavailability of herbal drug. J Pharm Biol Sci 3(1):51–57

44) Vu H, Hook S, Siqueira S, Müllertz A, Rades T, McDowell A (2018) Are phytosomes a superior nanodelivery system for the antioxidant rutin? Int J Pharm 548(1):82–91.

45) Singh R, Gangadharappa H, Mruthunjaya K (2018) Phytosome complexed with chitosan for gingerol delivery in the treatment of respiratory infection: in vitro and in vivo evaluation. Eur J Pharm Sci 122:214–229.

46) Merizzi G: An Antioxidant Preparation Based on Plant Extracts For Treatment of Circulation and Adiposity Problem, EP1214084, 2002.

47) Bombardelli E, Patri G, Pozzi R. Complexes of neolignane derivatives with phospholipids, the use thereof and pharmaceutical and cosmetic formulations containing them. Indena Spa, Milano, Italy 1992, Patent No. EP 0464297

48) Bombardelli E. Oral compositions for the treatment of cellulite. Indena Spa, Milano, Italy 2010, Patent No. US 7691422.

49) Gabetta B, Bombardelli, Ezio, Pifferi, Giorgio. Complexes of flavanolignanes with phospholipids, preparation thereof and associated pharmaceutical compositions. Inverni, Della Beffa Spa, Italy 1987, Patent No. EP 0209038

50) Giori A, Franceschi F. Phospholipid complexes of curcumin having improved bioavailability. Indena Spa, Milano, Italy 2007, Patent No. WO 2007/10155

51) Khare AB: Soluble Isoflavone Composition, WO/2004/045541, 2004

52) Bombardelli E, Patri, Gian Franco, Pozzi, Roberto. Complexes of saponins with phospholipids and pharmaceutical and cosmetic compositions containing them. Indena Spa, Milano, Italy 1988, Patent No. EP 0283713.

53) Franceshi F, Giori A: A Phospholipid Complex of Olive Fruits or Leaves Extracts Having Improved Bioavailability Patent No. EP1844785, 2007.

54) Bombardelli E, Mustich G. Bilobalide phospholipide complexes, their applications and formulations containing them. Indena Spa, Milano, Italy 1991, Patent No. EP 0441279.

55) Dipierro F: Composition Comprising Ginkgo Biloba Derivatives For Treatment of Asthmatic and Allergic Conditions Patent No. EP1813280, 2007.

56) Bombardelli E, Patri GF. Complex compounds of bioflavonoids with phospholipids, their preparation and use, and pharmaceutical and cosmetic compositions containing them. Indena Spa, Milano, Italy 1993, Patent No. EP 0275005.

57) Kleinman H, Goldstein A, Malinda K, Sosne G; Inventors. Treatment of skin, and wound repair, with thymosin beta 4. Google Patents. 2007.

58) Morazzoni P, Bombardelli E. Phospholipid complexes prepared from extracts of Vitis vinifera as anti-atherosclerotic agents. Google Patents. 2001

59) Doering T, Traeger A, Waldmann-Laue M. Cosmetic and dermatological composition for the treatment of aging or photodamaged skin. EP1640041. 2006.

60) Giori A, Franceschi F. Phospholipid complexes of curcumin having improved bioavailability. Google Patents. 2020

61) Habbu P, Madagundi S, Kulkarni R, Jadav S, Vanakudri R, Kulkarni V (2013) Preparation and evaluation of Bacopa-phospholipid complex for antiamnesic activity in rodents. Drug Invent Today 5(1):13–21.

62) Saoji S, Belgamwar V, Dharashivkar S, Rode A, Mack C, Dave V (2016) The study of the influence of formulation and process variables on the functional attributes of simvastatin–phospholipid complex. J Pharm Innov 11(3):264–278.

63) Li Y, Yang D, Chen S, Chen S, Chan A (2008) Process parameters and morphology in puerarin, phospholipids and their complex microparticles generation by supercritical antisolvent precipi tation. Int J Pharm 359(1–2):35–45.

64) Mascarella S (1993) Therapeutic and anti-lipoperoxidant effects of silybin-phosphatidylcholine complex in chronic liver disease. Curr Ther Res 53(1):98–102

65) Dasgupta T, Mello P, Bhattacharya D (2015) Spectroscopic & chromatographic methods for quan titative analysis of phospholipid complexes of flavonoids-a comparative study. Pharm Anal Acta 6(1):319–322.

66) Bombardelli E (1991) Phytosome: new cosmetic delivery system. Boll Chim Farm 130(1):431–438

67) Udapurkar PP, Bhusnure OG, Kamble SR (2018) Diosmin Phytosomes: development, optimiza tion and physicochemical characterization. Indian J Pharm Educ Res 52(4):S29–S36

68) Gnananath K, Sri Nataraj K, Ganga Rao B (2017) Phospholipid complex technique for superior bioavailability of phytoconstituents. Adv Pharm Bull 7(1):35–42.

69) Alexander, A., Ajazuddin, Patel, R.J., Saraf, S., Saraf, S. (2016) Recent expansion of pharmaceuti cal nanotechnologies and targeting strategies in the field of

phytopharmaceuticals for the delivery of herbal extracts and bioactives. J Control Release 10:110–124.

70) Kumari P, Singh N, Cheriyan B, Neelam J (2011) Phytosome: a noval approach for phytomedicine. Int J Inst Pharm Life Sci 1(2):89–100

71) Mamta B, Anar J, Shah R (2015) An overview of Ethosome as advanced herbal drug delivery system. Int J Res Rev Pharm Appl Sci 2(1):1–14

72) Chaturvedi M, Kumar M, Sinhal A, Saifi A (2015) Recent development in novel drug delivery systems of herbal drugs. Int J Green Pharm 5(2):87–94.

73) Kadu A, Apte M (2017) Phytosome : a novel approach to enhance the bioavailability of phytocon stituent. Asian J Pharm 5(2):453–461.

74) El-Menshawe S, Ali A, Rabeh M (2018) Nanosized soy phytosome-based thermogel as topical anti- obesity formulation: an approach for acceptable level of evidence of an effective novel herbal weight loss product. Int J Nanomedicine 13:307–318.

75) Rathee S, Kamboj A (2017) Optimization and development of antidiabetic phytosomes by box- benhken design. J Liposome Res 3(2):1–27.

76) Ochi M, Amoabediny G, Rezayat S, Akbarzadeh A, Ebrahimi B (2016) In vitro co-delivery evalu ation of novel pegylated nano-liposomal herbal drugs of silibinin and glycyrrhizic acid (nano- phytosome) to hepatocellular carcinoma cells. Cell J 18(2):135–148.

77) Abdelkader H, Longman M, Alany R, Pierscionek B (2016) Phytosome-hyaluronic acid systems for ocular delivery of L-carnosine. Int J Nanomedicine 11:2815–2827.

78) Mazumder A, Dwivedi A, Fox L, Brümmer A, Preez J, Gerber M (2016) In vitro skin permeation of sinigrin from its phytosome complex. J Pharm Pharmacol 68(12):1577–1583.

79) Angelico R, Ceglie A, Sacco P, Colafemmina G, Ripoli M, Mangia A (2014) Phyto-liposomes as nanoshuttles for water-insoluble silybin-phospholipid complex. Int J Pharm 471(1–2):173–181.

80) Avachat A, Patel V (2015) Self nanoemulsifying drug delivery system of stabilized ellagic acid-phospholipid complex with improved dissolution and permeability. Saudi Pharm J 23(3):276–289.

81) Direito R, Reis C, Roque L et al (2019) Phytosomes with persimmon (Diospyros kaki L.) extract: preparation and preliminary demonstration of in vivo tolerability. Pharmaceutics 11(6):196.