

# Phytosomes as Innovative Delivery Systems for Phytochemicals: A Comprehensive Review of Literature

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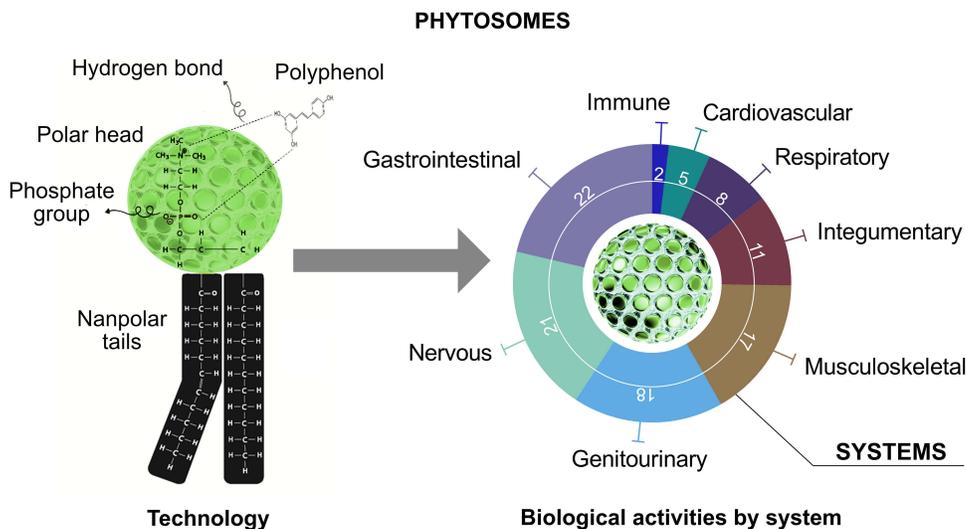
**Abstract:** Nowadays, medicinal herbs and their phytochemicals have emerged as a great therapeutic option for many disorders. However, poor bioavailability and selectivity might limit their clinical application. Therefore, bioavailability is considered a notable challenge to improve bio-efficacy in transporting dietary phytochemicals. Different methods have been proposed for generating effective carrier systems to enhance the bioavailability of phytochemicals. Among them, nano-vesicles have been introduced as promising candidates for the delivery of insoluble phytochemicals. Due to the easy preparation of the bilayer vesicles and their adaptability, they have been widely used and approved by the scientific literature. The first part of the review is focused on introducing phytosome technology as well as its applications, with emphasis on principles of formulations and characterization. The second part provides a wide overview of biological activities of commercial and non-commercial phytosomes, divided by systems and related pathologies. These results confirm the greater effectiveness of phytosomes, both in terms of biological activity or reduced dosage, highlighting curcumin and silymarin as the most formulated compounds. Finally, we describe the promising clinical and experimental findings regarding the applications of phytosomes. The conclusion of this study encourages the researchers to transfer their knowledge from laboratories to market, for a further development of these products.

**Keywords:** phytochemical, nanomedicine, phytosome, delivery, vesicle, disease

## Introduction

For several decades, medicinal herbs and their active constituents have been utilized to treat different diseases.<sup>1-5</sup> There are some major reasons for the increased use of herbal drugs: 1) modern medicine is unable to efficiently cure all the human pathologies, 2) there are increasing interests and attention over the assurance and safety of synthetic drugs, and 3) many natural products are being shown to produce better results than synthetic drugs without adverse effects.<sup>6</sup> However, due to poor oral bioavailability, the clinical application of numerous active compounds of plants is under debate.<sup>7,8</sup> The weak absorption rate of such constituents may be a result of low lipid solubility, the existence of multi rings polyphenols in their structures, and high molecular weight.<sup>9,10</sup> Different solutions have been suggested to face such obstacles,<sup>11</sup> including preparing emulsions,<sup>12</sup> liposomes,<sup>13</sup> and nano-formulation,<sup>14</sup> the adjustment of molecular structure,<sup>15</sup> and administration of prodrugs.<sup>16</sup> Between all approaches, phyto-phospholipid complexes (named phytosomes) are appeared to be a great method to boost their bioavailability.<sup>9</sup>

## Graphical Abstract



The term “Phyto” refers to the plant, while “some” refers to cell-like.<sup>17</sup> Phytosomes (or herbosomes) are the vesicular drug delivery system enhancing the absorption and bioavailability of low-soluble drugs.<sup>9,17</sup> Phytosomes are complex of phospholipids and natural active phytochemicals, bound in their structures, obtained by the reaction between phosphatidylcholine (or any hydrophilic polar head groups) and plant extracts in an aprotic solvent.<sup>10,18</sup> These formulations exhibit improved pharmacological and pharmacokinetic properties as compared to prevalent preparations. The lipid-soluble phosphatidyl portion completely covers the hydrophilic phytoconstituent-choline complexes. Phytosomes have remarkable benefits such as high drug encapsulation, reveal a better stability profile (chemical bonds are formed among the polar head of the amphiphile molecule and phytoconstituent<sup>19</sup>), and have a better bioavailability.<sup>20</sup> Moreover, a higher absorption rate leads to a lower dosage of active constituents for exerting a biological effect, also for polar phytoconstituents.

There is a variety of possible applications of phytosome that will be discussed in this review.

## The Phytochemicals

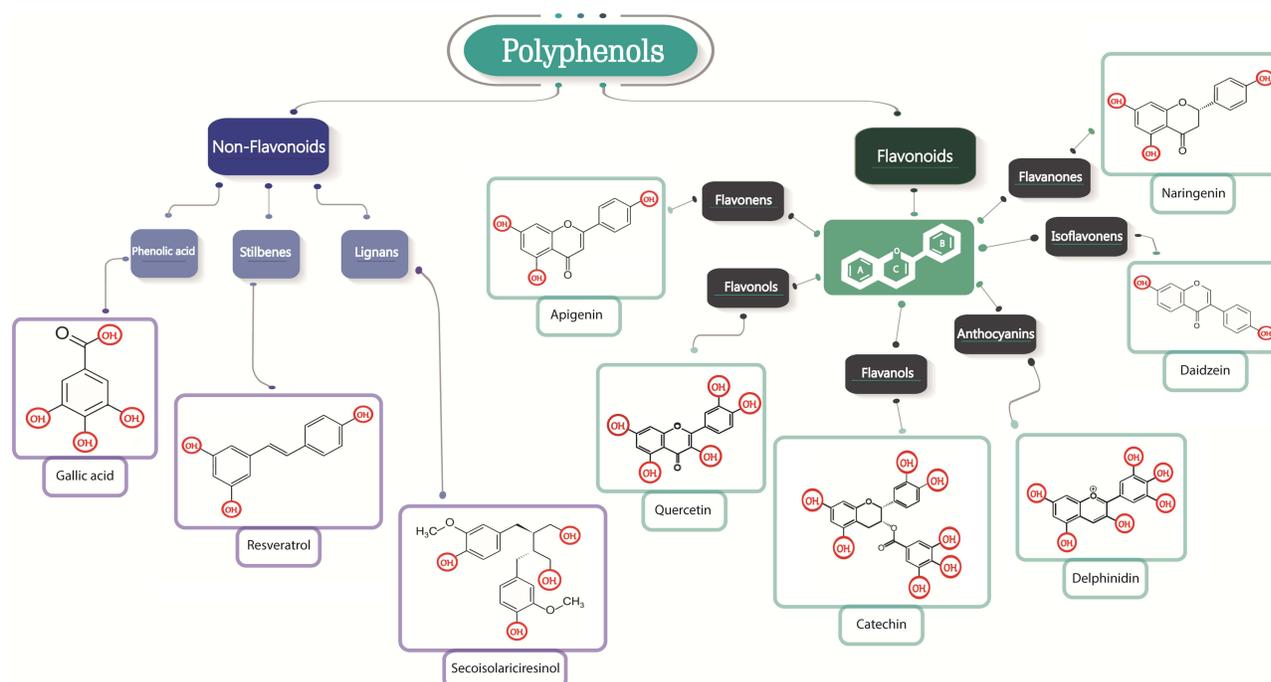
Phytochemicals or plant chemicals are comprised of a wide range of naturally occurring bioactive compounds produced by plants. The term bioactive refers to the ability of these compounds to interact with different components of living organisms, thereby exerting their beneficial effects. Phenolics, alkaloids, carbohydrates, lipids, terpenoids, and

other nitrogen-containing compounds are the most structurally different major categories of phytochemicals. Moreover, there are several subcategories of phytochemicals based on differences in biogenesis or biosynthetic pathway.

Between all the phytochemicals, only those having an active hydrogen atom (-COOH, -OH, -NH<sub>2</sub>, -NH, etc.), like polyphenols, can be integrated into a phytosome structure. An active hydrogen atom can form a hydrogen bond between the herbal derivatives and the hydrophilic parts of amphiphile molecules. Polyphenols are the major group of phytochemicals extensively found in plant-based foods. Potential health effects of polyphenols were shown in different diseases including cancer, inflammation, neurodegenerative and cardiovascular diseases, type 2 diabetes, and obesity.<sup>21</sup> Essentially, they are found in conjugated forms composed of sugar residues (one or more) attached to hydroxyl groups; however, the sugar residues may directly attach to an aromatic carbon.<sup>22,23</sup> Flavonoids and non-flavonoids are two major subgroups of polyphenols (Figure 1). The current review updates the knowledge on the use of polyphenols through phytosomes, paying attention to their structure, preparation, and the biological activities associated with the use of phytochemicals-loaded phytosome.

## Phytosome Structure and Preparation Methods

Bombardelli et al stated for the first time that there is a chemical bond between phospholipids and flavonoid vegetal



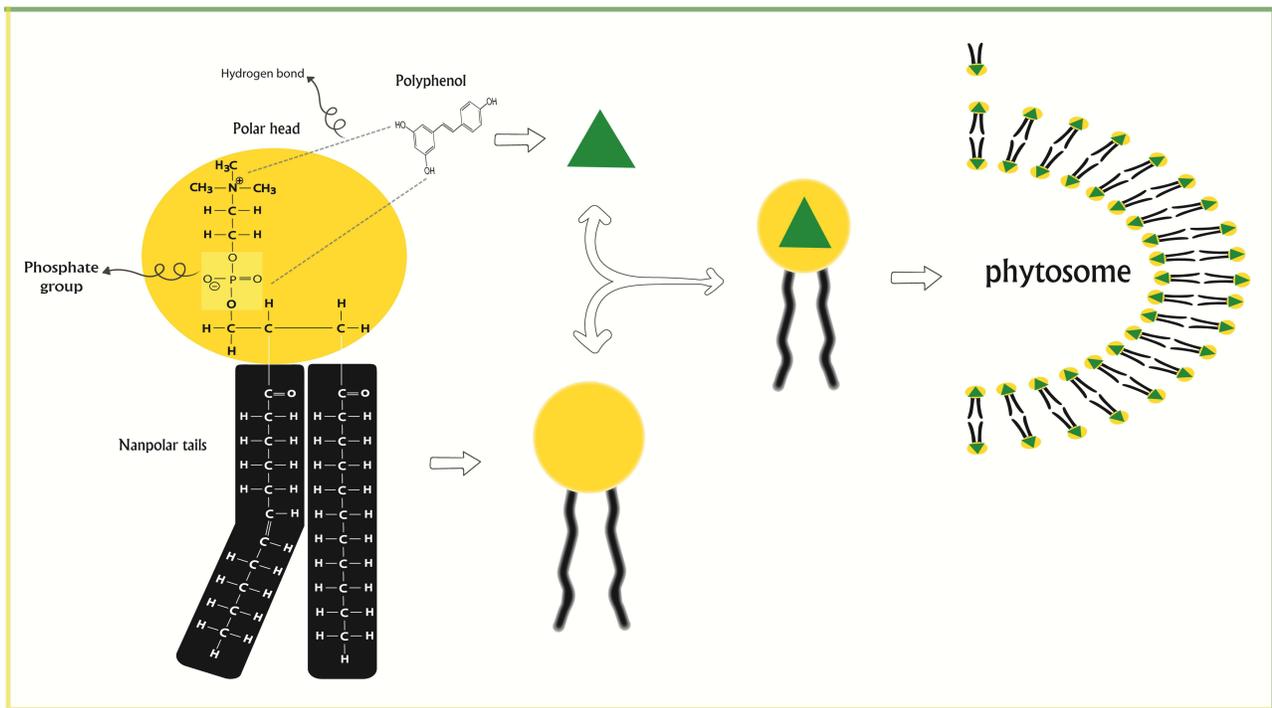
**Figure 1** Polyphenol classifications. Classes of polyphenols and their relationships to each other. One structural example is presented for each class.

derivative molecules.<sup>24</sup> In 2016, Pu et al examined the molecular docking model for the interaction of 20(S)-protopanaxadiol (PPD) phospholipid complexes. The results indicated that the hydrophobic section of the PPD framework was enclosed by two hydrophobic arms of the phospholipid molecule, and a hydrogen-bond with the phospholipid backbone of the P=O section was generated by one of the hydrophilic-OH groups. Many authors have stated that the hydrogen interactions are the main interactions in phytosome vesicles.<sup>25</sup>

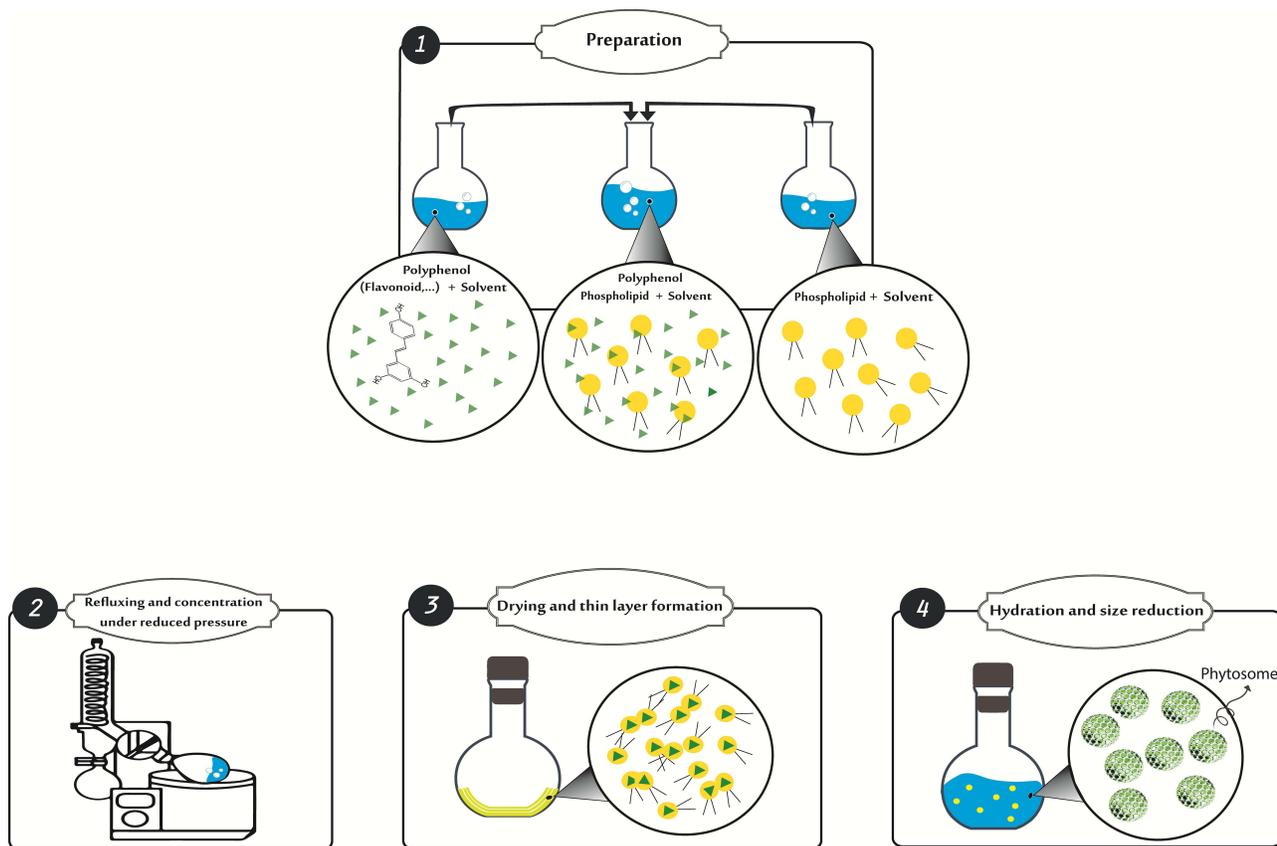
Phospholipids have an affinity for polyphenols and form supramolecular adducts that have a definite stoichiometry, which can be obtained from thermal analysis. Semalty et al tested this parameter and found that hydrogen bond formation or hydrophobic interactions were due to the interaction between the two molecules.<sup>26</sup> The phospholipid-active ingredient is responsible for the creation of a hydrogen connection between the polar head and the active ingredient's polar functionalities.<sup>25,27</sup> In summary, as presented in Figure 2, the hydroxyl groups of polyphenols can interact effectively with nitrate and phosphate groups of phospholipids.

Several strategies have been proposed for preparing phytosome, such as the rotary evaporator method, anti-solvent precipitation technique, freeze-drying co-solvency, and salting-out technique. The main methodologies for the preparation of the phytosome are shown in Figure 3. Popular and

commonly used techniques for producing phospholipid complexes are the evaporator approach and solvent evaporation. The solvent evaporation method for preparing evodiamine phospholipids complex was stated by Liu et al.<sup>28</sup> In another study, Yu et al prepared the berberine-loaded phytosomes by the method of solvent evaporation and a self-assembly approach.<sup>29</sup> In the process of solvent evaporation, lipid materials were dissolved in an organic solvent, which was then removed by vacuum rotary evaporation. By the anti-solvent precipitation technique, Singh et al reported the preparation of lawsone-loaded phytosome.<sup>30</sup> In this process, dichloromethane was refluxed with lawsone and soya lecithin at a temperature not exceeding 60 °C. Then, to get the precipitate stored overnight in vacuum desiccators, n-hexane was added. Karole et al have used the technique of anti-solvent precipitation to prepare phytosomes containing *Bombax ceiba* extract.<sup>31</sup> El-Menshawe et al described a soy thermogel based on phytosome made by three different preparation methods (co-solvency, solvent evaporation, and salting-out).<sup>32</sup> It was observed that the optimal phytosome formulation was the one prepared using the co-solvency technique, obtaining an ideal entrapment efficiency (EE) of 99.89%, a size of 64.44 nm, and a release rate of up to 93% after 2 hours. Demir et al developed a novel liposomal formulation in an innovative study by encapsulating both *Calendula officinalis* extract and AuNPs.<sup>33</sup> Vesicle preparation was



**Figure 2** Suggested principle for phytosome formation. Hydrogen bond formation between phytochemical and polar head of phospholipid is depicted as schematic and structural picture. Dashed lines are representative as the hydrogen bonds.



**Figure 3** Thin-film hydration as the most common method for phytosome preparation. Steps 1 to 4 are the procedures of phytosome preparation.

carried out by the conventional method of thin-film hydration within the extrusion. The findings showed that this method improved the biological activity of AuNP and calendula extract. Other methods have been documented for the preparation of phytosome complexes, such as anhydrous co-solvent lyophilization or lyophilization.<sup>34–36</sup>

Phytosomes are originated from the reaction of a stoichiometric quantity of the phospholipid (phosphatidylcholine) with polyphenolic constituents or standardized extracts (flavonoids, tannins, terpenoids, xanthenes) within a non-polar solvent.<sup>37</sup> Different solvents have been used in various studies as a reaction medium to formulate phyto-phospholipid complexes. In aprotic solvents, no hydrogen atoms exist directly connected to an electronegative atom and have no capability at hydrogen bonding. Traditionally, these solvents like aromatic hydrocarbons, methylene chloride, halogen derivatives, cyclic ethers, and ethyl acetate have been utilized for preparing phyto-phospholipid complexes. However, these are mostly substituted by protic solvents, such as ethanol.<sup>38,39</sup> In protic solvents, like methanol and ethanol, at least one hydrogen atom is directly connected to an electronegative atom. Thanks to the higher yield of complexes, ethanol is an effective solvent also due to the low presence of residues. Some liposomal drug complexes act in the existence of buffer solution or water, where the interaction of the phytosomes with a solvent occurs with a decreased dielectric constant.<sup>40</sup> Nevertheless, the use of a single solvent is included in most preparation methods, mixed solvent systems have been used in several studies whereby the phospholipids are dissolved in a different solvent from that of the drug/extract. The mixed solvent systems include dichloromethane and methanol, water and diethyl ether, as well as ethanol and dichloromethane.<sup>41–43</sup>

## Vesicular Systems in Phytosome Development

Targeted delivery and sustained release rate are two relevant factors for phytochemical drug carriers.<sup>44</sup> Several kinds of nano-systems would be used in various disease imaging or therapies, or as theranostics.<sup>45</sup> The most used nanocarriers for phytochemicals are the vesicular drug delivery systems,<sup>46</sup> in which active compounds are encapsulated in a spherical structure.<sup>47</sup>

Various types of vesicular drug delivery systems such as liposome, niosome, transfersome, and ethosome have been developed (Table 1).<sup>48–65</sup> Figure 4 also depicts a schematic representation of the different vesicle architectures in phytochemical delivery.

## The Liposome

Liposome originated from two Greek words “Lipos signifying fat and Soma meaning body”.<sup>67</sup> Liposomes are phospholipids and cholesterol that made up the spherical shaped vesicles with a diameter of 0.05–5.0 micrometers. They are a very promising carrier for drug delivery in different architectures due to their hydrophobic and lipophilic characters.<sup>68–70</sup> This drug delivery system attempts to directly target the drug at the desired site of action.<sup>71</sup> Liposomes are biocompatible, biodegradable, stable, and have a unique property that traps both hydrophilic and lipophilic agents into their compartments and provides a controlled-release effect.<sup>72–74</sup> Liposomes are used in different pathological conditions, such as cancer, inflammation, eye and skin disease, malaria, and osteosarcomas.<sup>75–80</sup> The liposomes can be designed using various techniques.<sup>81,82</sup> Overall, most liposome preparatory methods are based on (1) solvation of the lipids in an organic solvent; (2) getting lipid thin film by evaporation; (3) hydration of lipid layer by a hydrophilic solvent; (4) liposome purification (5) and characterize the properties of the final liposome.<sup>83–85</sup> Also, other synthesis methods can improve the encapsulation of the loaded drug.<sup>86–88</sup>

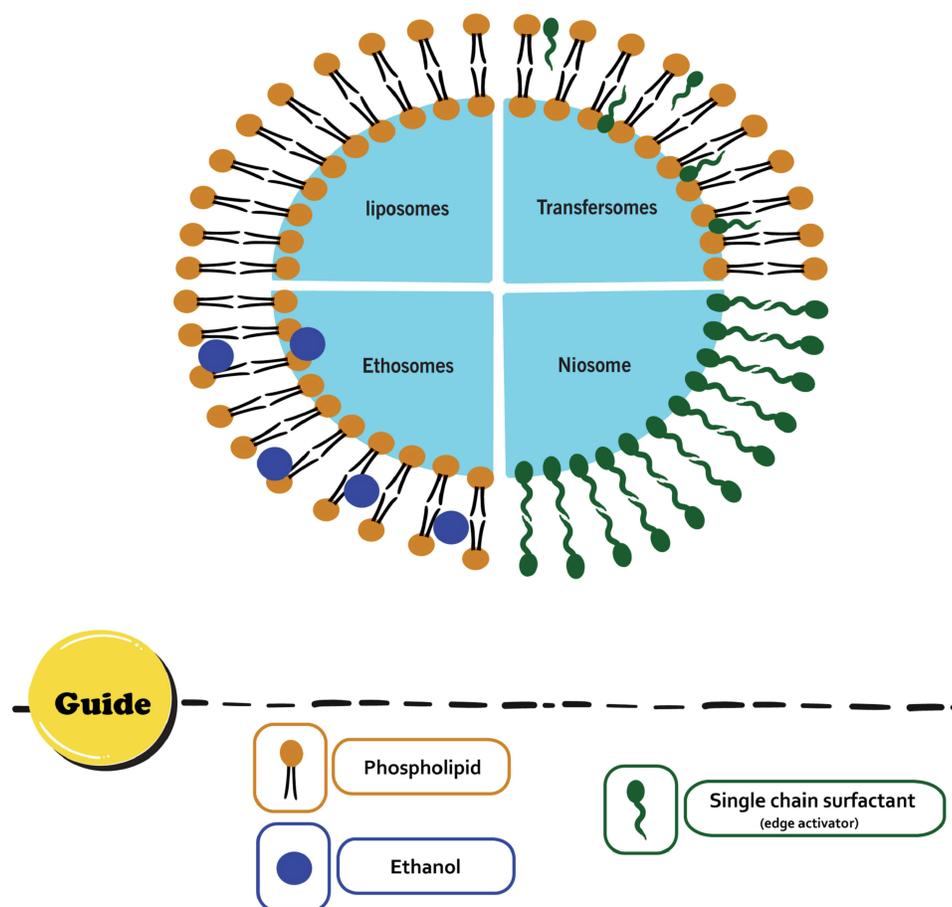
Besides, phytoconstituent liposomes have been developed to increase the penetration, solubility, and biological impact or to defend against degradation.<sup>89,90</sup> There are many reports of the use of natural extracts via encapsulation in liposomes to improve their bioactivity or to avoid other side effects.<sup>91,92</sup> For example, Gautam et al reported CD44 receptor-phyto-liposomes loaded with stigmasterol (STS) for synergistic chemotherapy. The *in vitro* anticancer activity of HA-DOX-STS-lipo was significantly enhanced in MDA-MB-231, CD44-overexpressing cells relative to MCF-7 cells demonstrating HA-mediated targeting effect. HA-DOX-STS-lipo accumulated more and increased antitumor efficacy in the MDA-MB-231 xenograft tumor model expressing high levels of CD44, suggesting the potential of carrier system toward CD44-overexpressing tumors.<sup>93</sup> Rafiee et al prepared nanoliposomes using a thin hydration process with various amounts of polyphenols of pistachio green hull extract and lecithin and characterized their particle size, PDI, zeta potential, entrapment efficiency (EE), and morphology. Nanoliposomes had the highest EE (52.93%) composed of 1% lecithin with 1000 ppm phenolic compounds. The FTIR findings show the formation of hydrogen bonds between both the phospholipid polar zone and

**Table 1** Most Used Nanovesicle Encapsulated Herbal Formulations

Nanovesicle	Phytochemicals	Feature	References
Liposome	<i>Aphanamixis polystachya</i> leaf	Great improvement in memory function, locomotive behavior, and dementia-induced outpatient quality of mice.	[63]
	Anthocyanins	Increase physiological stability in vitro for 14 days and increase the activity of ROS scavenging and skin absorption.	[62]
	Curcumin	Fast permeation rate endothelial cell monolayer crossing blood-brain barrier (BBB) and good durability toward digestive enzymes.	[61]
	<i>Eleusine coracana</i>	Effective antibacterial formulations have a great nutritive value.	[60]
Niosome	<i>Carum carvi</i>	Regulate release and decrease of MCF-7 cell migration, high anti-cancer behavior against MCF-7 supported by cytometry flow (G2/M arrest).	[59]
	Lawson	Entrapment efficiently of 70%, a sustained release profile, and a significant increase in antitumor activity.	[58]
	<i>Fumaria officinalis</i>	Rapid degradation, stability in GI conditions simulated, and anti-diabetic and anti-inflammatory capacity.	[57]
	<i>Annona squamosa</i>	Aid with topical drug enhancers to purify the body from harmful impurities and oxidants and can be applied directly to the skin.	[56]
Transfersome	<i>Mulberry leaves</i>	Prolonged delivery system, strong safety, and acne vulgaris care via transdermal route.	[55]
	Apigenin	Drug entrapment of 84.24%, strong stability, enhances the permeability of apigenin in the long-term release.	[53]
	Epigallocatechin-3-gallate (EGCG)	Increases cell viability, decreases lipid peroxidation, intracellular ROS, MMP expression in HaCaT cells, and increases skin permeation.	[52]
	Emodin	High efficiency and stability in encapsulation, reduces body weight, and adipocyte size through ATGL up-regulation, down-regulation of G0S2 expression in adipose tissue, and improved insulin sensitivity.	[51]
Ethosome	Thymoquinone	99% efficiency for drug trapping. Cytotoxic activity of 0.95 µg/mL against MCF-7 cell lines is improved.	[54]
	Capsaicin	Ethosomal hydrogels improve performance and patient compliance with capsaicin treatment.	[50]
	<i>Terminalia chebula</i>	Effective release comparison with extract in the gel. In vitro anti-arthritis activity demonstrates important anti-arthritis activity as opposed to normal Diclofenac activity	[49]
	Paeonol	Paeonol-loaded ethosomes showed improved transdermal absorption and skin retention (138.58 µg/cm <sup>2</sup> and 52.60 µg/cm <sup>2</sup> , respectively)	[66]

the phenolic compound OH groups. Also, nanoliposomes obtained a significant shelf life. As a result of this study, the liposome can be used as an effective carrier for the maintenance and enhancement of pistachio extract and bio-functional active agents in food products.<sup>94</sup> In another study, Shafaei et al evaluated the therapeutic efficacy of sinensetin (SIN), eupatorin (EUP), rosmarinic acid (RA), and 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF) in *Orthosiphon stamineus* extract (OS-E) and assessed the formulation of OS-E-derived nanoliposomes (OS-EL) in

the plasma of Sprague-Dawley rat after oral and intravenous administration. After intravenous OS-EL administration, all four compounds tended to be poorly distributed and gradually removed from the body as opposed to OS-E. On the other hand, in oral administration loaded formulation (OS-EL), the bioavailability of all compounds was greater than OS-E (due to higher solubility of phospholipid encapsulation). These findings indicate that OS-EL's greater solubility and bioavailability may be due to liposome encapsulation.<sup>95</sup>



**Figure 4** Possible vesicles to form phytosomes. Schematic representation of the different types of vesicles in phytochemical delivery, liposome, transfersome, niosome and ethosome. All these possible vesicles have polar heads.

In a most recent study, Sinisgalli et al evaluated the antioxidant activity of *Capsicum annuum* pepper extract-loaded liposomes. The extracts exhibited no cytotoxicity and reduced the level of ROS in the HepG2 cell line. Based on the RT-PCR assay, the expression of endogenous antioxidants was increased in loaded formulations.<sup>96</sup> Besides the enhanced ability in phytochemical delivery, liposomes have also some disadvantages. Drugs encapsulated in the liposomes require a high cost of development. Leakage and fusion of encapsulated drugs may occur. Furthermore, the phospholipid liposome may undergo hydrolysis and oxidation, resulting in a shorter half-life.

## The Niosome

Niosomes are nanometric lamellar vesicles that are formed by combining non-ionic surfactant and a helper lipid-like cholesterol.<sup>97-99</sup> The non-ionic surfactants create a stable bilayer vesicle in hydrophilic systems by using energy (physical agitation and heating).<sup>100,101</sup> Hydrophobic parts in the

bilayer structure are guided aside from the aqueous phase, while the hydrophilic heads stay in contact with the aqueous side. The surfactants used in the preparation of niosomes should be biocompatible, biodegradable, and not immunogenic.<sup>102,103</sup> Niosomes act like liposomes in vivo and in vitro, extending the circulation of the encapsulated phytochemical, adjusting its organ distribution, and improving bioavailability. The niosomal formulations are more leaky than liposomes with the same cholesterol value.<sup>99</sup> Previous research has been shown that cholesterol concentration is an important influence factor on vesicle leakage.<sup>104</sup> As a result, the efficiency of liposomal drug trapping becomes lower than niosomes.<sup>105</sup> Liposomes are expensive, and their components are unstable for long periods and need special handling and storage.<sup>106</sup> Niosomes can increase the solubility and sustainability of phytochemicals, considered novel herbal delivery systems. They are designed to target and control the release of natural compounds.<sup>57,107-110</sup> Our group evaluated the niosome encapsulation of different antioxidant phytochemicals,

such as lawsone,<sup>58</sup> diosgenin,<sup>111</sup> D-limonene,<sup>111</sup> and Carum spp.<sup>111</sup> In our last study, we designed a natural anti-cancer niosome vesicle based on ergosterol, nonionic surfactants, and Carum carvi extract (Carum). In vitro cytotoxicity, flow cytometry, DNA fragmentation, and cell migration assay of formulations were evaluated. Loaded formulations provided a controlled release compared with free Carum extract. Based on MTT assay and flow cytometry analysis for MCF-7 cancer cell line, Carum encapsulated niosome (Nio/Carum) showed better anti-cancer effects than free Carum extract. Cell cycle analysis showed G2/M arrest in Nio/Carum formulations. Nio/Carum remarkably decreased the migration of MCF7 cells.<sup>111</sup>

Similarly, to improve solubility, stability, and penetration of antioxidant flavonoids (morin, quercetin, myricetin, fisetin, rutin, and breviscapine), Lu et al loaded these phytochemicals into niosome. Results revealed that quercetin showed significant whitening and antioxidant potential and the loaded niosome forms a spherical shape with a size of 97 nm, 31.1 mV zeta potential, and 87.3% drug trapping efficiency.<sup>336</sup> Rabia et al reported an in vitro assessment of the nanovesicles containing marigold extract and called it phyto-niosome.<sup>112</sup> Their results showed marigold and its entrapped form in a surfactant-based delivery vesicle have a promising potential for different bio-applications as well as food, its possible use as a component for food additives and dermal cosmetic formulations. Niosomes greatly increased the bioavailability and photostability of quercetin. Quercetin-loaded niosomes had a prolonged-release, increased transdermal absorption, and skin absorption 2.95 times stronger than quercetin solution.<sup>113</sup> Niosomes have some additional advantages over liposomes but also showed some disadvantages. Component of niosome (non-ionic surfactants) is not generally recognized as safe (as phospholipid in liposomes). They are indeed more irritant than liposomes.<sup>114</sup> Table 1 reports some examples of phytochemical-encapsulated niosomes.

## The Transfersome

Transfersomes are a type of deformable or elastic nanocarriers that were first emerged in the early 1990s.<sup>64</sup> The regular liposomes do not permeate into the layers of the skin and remain confined to the outer stratum corneum layer (Figure 4).<sup>115</sup> Therefore, new types of lipid vesicles such as transfersomes have been constructed as an improved type of liposomes. Transfersome is an elastic and ultra-flexible lipid carrier with highly deformable membranes that enhance the transfer of compounds to deeper skin tissues.<sup>116</sup> The transfersome consists of at least one amphipathic molecule (soy

phosphatidylcholine) and a bilayer softening agent for vesicle flexibility (generally a surfactant). When transfersome components are applied to aqueous systems, they self-assembled into a lipid bilayer that finally closes into a lipid vesicle.<sup>116</sup> Studies of penetration and deformability have shown that transfersomes give deeper penetration of the skin. Transfersome can be used as medication carriers for peptides, small molecules, proteins, and particularly herbal components.<sup>117</sup> In a recent paper, Wu et al prepared resveratrol (RSV) loaded transfersomes consisting of the liposomal system phosphatidylcholine (PC) and the non-ionic edge stimulators (EA).<sup>337</sup> Results showed that a 5% ethanol and 5% PC/EA (3:1) in distilled water could make the optimum formulation. The size of vesicles was 40 nm, and the EE% was 60%. Based on antioxidant activity results, the transfersomes were nearly equivalent to the RSV (free RSV) group. Also, the D1-20(W) formulation showed an improvement of 27% accumulation for in vitro transdermal delivery analysis. Cell viability analysis revealed that D3-80(W) cytotoxicity was decreased by 34.45% compared to the free RSV.<sup>118</sup> Because of their susceptibility to oxidative stress, transfersomes are not chemically stable. The purity of natural phospholipids is also another factor that limits the adoption of transfersomes as standard vehicles for the delivery of drugs. On the other hand, transfersomes can be synthesized on a large scale with simple and easy processes, without the use of pharmaceutically unsuitable additives.<sup>64</sup> More examples of herbal loaded transfersome are shown in Table 1.

## The Ethosome

Ethosomes are non-invasive carriers that allow medicinal products to enter deep skin layers and systemic circulation.<sup>119</sup> Ethosomes are soft vesicles customized to improve the delivery of active agents, such as drugs and natural products. They are primarily composed of phospholipids (phosphatidylserine, phosphatidylcholine, and phosphatidic acid), high ethanol concentrations, and deionized water.<sup>120</sup> The high concentration of ethanol makes ethosomes the best choice for skin due to impairment of the skin lipid bilayer. Thus, when ethanol is incorporated into the vesicle membrane, it provides the ability to reach vesicles to the stratum corneum. The lipid membrane in ethosomes is also packaged less firmly than other vesicles due to the presence of ethanol and this ability results in improved drug trafficking capability in stratum corneum lipids.<sup>121</sup> The ethosomes showed to be appropriate in the biotechnology, pharmaceutical, cosmetic, veterinary, and nutraceutical industries for different purposes. Therefore, these soft vesicles serve as

new vesicular carriers for improved skin delivery.<sup>122</sup> The size of ethosomes may be modified from nanometers to micrometers. Ethosomes have been found to be significantly superior in the quantity and depth of drugs delivered through the skin compared to liposomes and many other commercial transdermal and dermal delivery platforms.<sup>123</sup> A comparative evaluation of phytosome, liposome, niosome, ethosome, and transfersome in nano-delivery systems is summarized in Table 2.

Many authors have shown the advantages of ethosomes as topical vehicles of phytochemicals. Sasindran et al examined the cytotoxicity of combined herbal extracts (*Zingiber officinale*, *Croton tiglium*, and *Phyllanthus niruri*) and extracts loaded ethosome for transdermal delivery. Results of the cell-line analysis indicated that ethosomes loaded with extract inhibit testosterone and improve cell viability similarly to the standard drug (minoxidil). Even so, the encapsulated vesicle did not harm the rat skin layer (based on histopathological study).<sup>124</sup> The drawback of ethosome is the size variation from nanometers to micrometers, due to its poor consistency and evaporation of ethanol, which leaks out from loaded compounds after a while. To manage this deficiency, alcohol can be located with a combination of propylene glycol and trehalose.<sup>125</sup> Summarized examples of phospholipids and surfactants that are used in liposome, niosome, ethosome, and transfersome preparations are presented in Figure 5. All mentioned vesicular systems could be used in phytosome technology according to their applications.

## Phytosome Characterization

Nanomaterial measurement approaches are a rapidly growing field, involving effective methods for physical and chemical characterization.<sup>126</sup> Phytosomes have received tremendous attention for phytochemical delivery as a fast-growing class of nanovesicles. Several techniques were employed to characterize phytosomes size, elemental composition, morphology, and a wide range of other physical characteristics. There are physical properties, which can be investigated by more than one technique. Different limitations and strengths affect the choice of the most appropriate method, while a combinational methodology for characterization is often required.<sup>127</sup> Also, some statistical studies are needed for better application in real world.<sup>128,129</sup> The main characteristics of phytosomes are (1) size and shape; (2) surface charge; (3) chemical composition; (4) lamellarity and stability; (5) encapsulation efficiency and (6) release behavior. The goal of this

chapter is to provide a thorough summary and a systematic overview of all analytical instruments used to characterize phytosomes, including the latest papers.

## Average Size and Shape

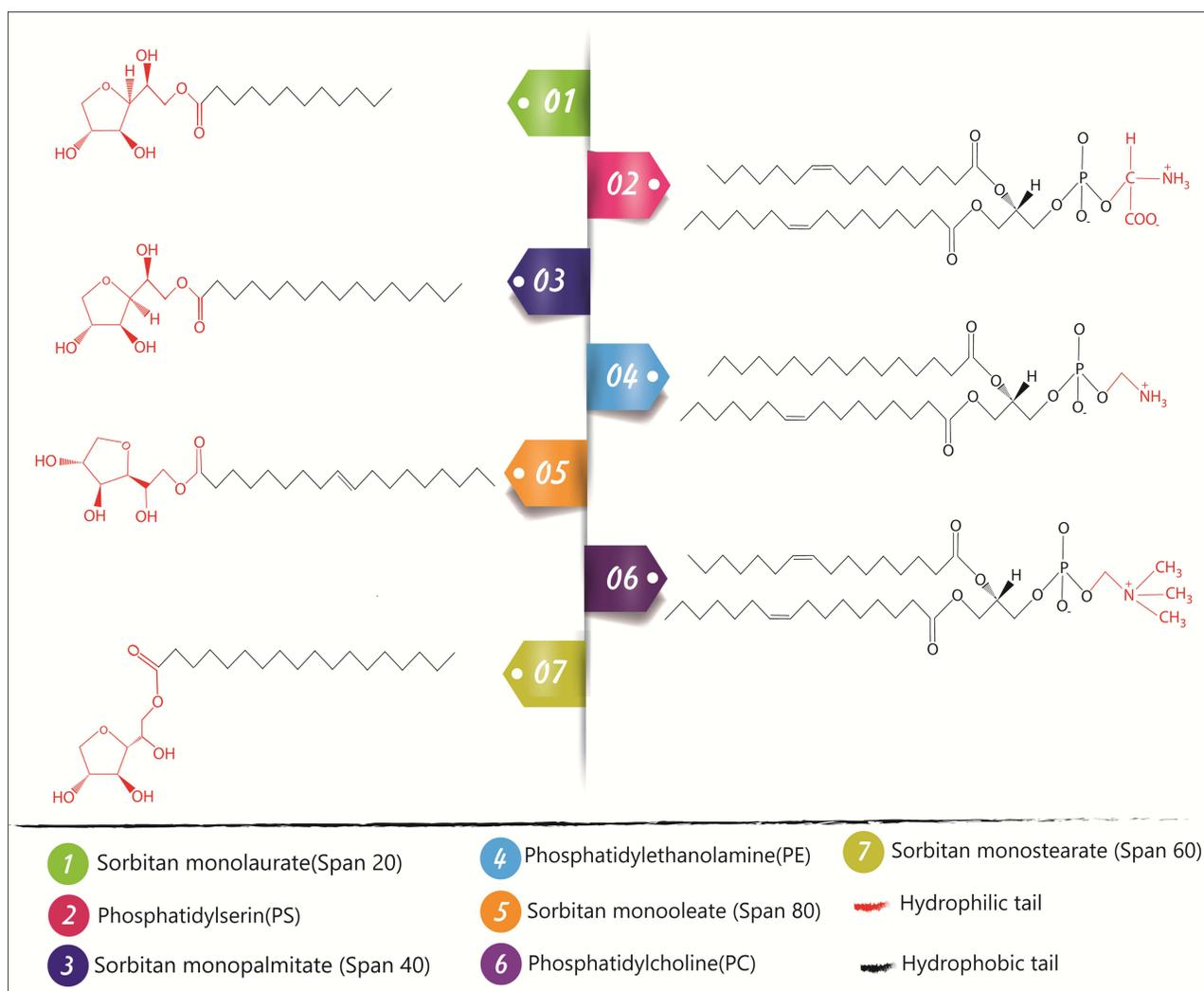
The evaluation of size and morphology is a critical phytosome analysis and provides valuable insight into the quality and different forms of a sample. Different techniques such as DLS,<sup>130</sup> microscopic observation<sup>131</sup> (TEM, SEM,<sup>132</sup> optical,<sup>133</sup> atomic force,<sup>134</sup> fluorescence,<sup>135</sup> etc.), and flow<sup>136</sup> and size-exclusion chromatography<sup>137</sup> can be used for phytosome size characterization. Electron microscopy is broadly used for phytosome visualization, and Cryo-TEM and Freeze-fracture-TEM are the most used.<sup>138</sup> Cryo-TEM could show phytosomes directly in the frozen state to prevent phytosomal disruption.<sup>139</sup> Freeze-fracture-TEM provides the details on liposomal size and morphology without any structural distortion.<sup>140</sup> Methods of microscopy are generally of high resolution and rapid productivity, but the sample preparation is complicated and time-consuming; also, some problems such as shrinking or shape distortion can be generated in sample preparation.<sup>141</sup> The measurement of phytosome size distribution and polydispersity gives data on their physical stability, which can be evaluated by DLS.<sup>142</sup> DLS is easy, precise, accurate, very fast and can therefore be used for regular size distribution measurements of phytosomes.<sup>143,144</sup> The biggest benefit of DLS is that the assessment could be carried out in the sample's natural environment.<sup>145</sup> The disadvantage of this approach is that the heterogeneous emulsions could result in false data.<sup>146</sup>

## Surface Charge

Zeta potential (complete charge generated by medium) defines the charge of phytosomes in emulsions. Zeta potential may be negative, positive, or neutral depending on the composition of the phytosome.<sup>147,148</sup> Zeta potential could reflect the stability of phytosomes in a medium; in fact, charged particles repel each other enough to maintain stability. Phytosome emulsion with a zeta potential greater than or less than 30 mV is known to be stable.<sup>149</sup> The electrostatic properties of phytosomes can be measured using Doppler velocimetry,<sup>150</sup> zeta sizer,<sup>151</sup> master size,<sup>152</sup> microelectrophoresis,<sup>153</sup> pH-sensitive fluorophores,<sup>154</sup> high-performance capillary electrophoresis,<sup>147</sup> and DLS instruments.<sup>155</sup> Laser Doppler velocimetry is the method for measuring the velocity or linear or vibrational motion of phytosome emulsions using the Doppler Effect in a laser beam. In light-scattering methods, an electrical field

**Table 2** Comparative Evaluation of Phytosome, Liposomes, Niosome, Ethosome and Transfersomes in Nano-Delivery Systems

Characteristics	Phytosome	Liposome	Niosome	Ethosome	Transfersome
Composition	Phospholipids and polyphenolic phytoconstituents	Phospholipids and cholesterol	Non-ionic surfactant and cholesterol	Phospholipid, alcohol, polyglycol and water	Phospholipids and surfactant mixture
Flexibility	Rigid	Rigid	Rigid	Elasticity	Ultra-deformable
Main application	Phyto-delivery	Drug and gene delivery	Drug delivery and cosmetics	Skin delivery	Skin delivery
Administration	Oral, parenteral topical, transdermal	Oral, parenteral topical, transdermal	Oral, parenteral topical, transdermal	Topical and transdermal	Topical and 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	Improved dispersion of compounds with solubility issues, high stability, low-cost materials	Enhance permeation of drugs across/through the skin in an efficient manner	High deforming ability which ensures deeper penetration in skin layers
Limitations	Leaching of the phytoconstituents which reduces the desired drug concentration indicating their unstable nature	Low skin penetration, low stability	Low skin penetration and toxicity of surfactant	Poor yield, coalescence and fall apart on transfer into water, Loss of product during transfer form organic to water media	Toxic effect of surfactant
Marketed Product	Leucoselect, Greenselect, Panax ginseng, Sabalselect, etc.	Doxil, Abelcet, Visudyne, DepoDur, etc.	Lancome and L'Oreal	MaccabiCARE, Nanominox, Trima, etc.	Daktarin



**Figure 5** Amphiphile molecules in phytosomes. The most amphiphile molecules used in vesicle preparation in liposome, niosome, ethosome, and transfersome. Red parts are polar and black parts are non-polar.

is applied to the cell that causes phytosome movement within the cell. The results of the size were obtained from these movements of particles.

## Chemical Composition

Assessment of the chemical composition and interaction between vesicle components and phytochemicals is usually studied by NMR,<sup>156,157</sup> FTIR,<sup>158</sup> and mass spectrometry.<sup>159</sup> Besides, phospholipid quantification in phytosomes can be done by reaction with an appropriate reagent, followed by a spectrophotometric quantification.<sup>160</sup> Due to high signal-to-noise, sensitivity, and selectivity, mass spectrometry is one of the most credible techniques for determining the phytochemical composition of plant extracts and phospholipids.<sup>113</sup> Many authors have also applied FTIR techniques to determine the interaction between phytochemicals and vesicle components.

For example, de Azambuja Borges et al evaluated the interaction between soy isoflavone genistein and asolectin-loaded liposomes by HATR-FTIR, high-field <sup>31</sup>P NMR, and low-field <sup>1</sup>H NMR methods. The findings showed that isoflavone reduces the phosphate group's degree of hydration and mobility.<sup>161</sup> In another study, Mazumder et al confirmed that DSC and FTIR can prove the formation of the sinigrin-phytosome complex.<sup>35</sup> Chen et al also prepared curcumin-liposomes, and TGA and FTIR showed a successful presence of SA and PSA in liposomal lipid bilayers and covalent bonding between SA carboxyl group and WGA amine group.<sup>61</sup>

## Lamellarity and Stability

The word “lamellarity” represents the number of phytosomal lipid bilayers.<sup>162</sup> The most used methods for the determination of lamellarity are electron microscopy methods, <sup>31</sup>P nuclear

magnetic resonance, and small-angle X-ray scattering.  $^{31}\text{P}$  NMR is one of the most precise and simple methods for determining the lamellarity. The weakness of this approach is that it is sensitive to experimental conditions, such as the concentration of the reagent, vesicle type, and concentration of the buffer. Other recently applied visualization methods are negative staining electron microscopy, freeze-fracture, and cryo-microscopy. In order to evaluate the architecture of the vesicle membrane, Nele et al recently merged cryogenic transmission electron microscopy and small-angle neutron dispersion and offered insights into the impact of the formulation method and lipid composition on the development of liposomes with a defined membrane structure.<sup>163</sup>

Phytosomal stability is another important factor in the successful design of a successful carrier. Studies of stability are performed to explore the phytochemical changes of phytosomes during storage and general circulation. Stability can be assessed over several months by determining mean vesicle size, zeta potential, size distribution, and trap efficiency. Cheng et al assessed the thermal and photochemical stability of rhamnolipids (RL) modified curcumin liposomes and results showed improved stability of the loaded liposomes at different pH, ionic, and heat conditions.<sup>118</sup>

## Encapsulation Efficiency and Release Behavior

Encapsulation efficiency (EE percent) describes the amount of phytochemical that is embedded in the phytosome. EE percent can be described as equation 1:

$$EE\% = \frac{IP - EP}{IP} \times 100 \quad (1)$$

where EE% is the efficiency of encapsulation, EP is encapsulated phytochemical and IP is the initial content of phytochemicals.

The process of encapsulation efficiency determination begins with the removal of free unencapsulated phytochemicals from the phytosome emulsion by the Sephadex gel column, ultracentrifugation, or dialysis method (defined cut-off) for several hours against buffer solution. Step 2 in EE estimation is the ruination of the phytosome bilayer (with Triton X-100, acetonitrile, methanol, and ethanol) and the quantification of the released active agent by different methods, such as enzymatic assays, gel electrophoresis, fluorescence spectroscopy, and field flow fractionation chromatographic methods, such as HPLC, UPLC, or LC-MS.

Drug release behavior of vesicle carriers has been the subject of extensive research over the past few years, since the release profile obtained in vitro may provide an indicator of the efficiency of the carrier in vivo.<sup>164</sup> Membrane diffusion strategies (dialysis, micro-dialysis, fractional dialysis, and reverse dialysis), sample and separate strategy, in situ process, and continuous flow are traditional approaches that are most widely used to determine the release rate of active agents.<sup>165–170</sup> Phytochemical release can be spectrophotometrically determined. Table 3 shows a summary of the experimental techniques that can be used for the characterization of the phytosomes.

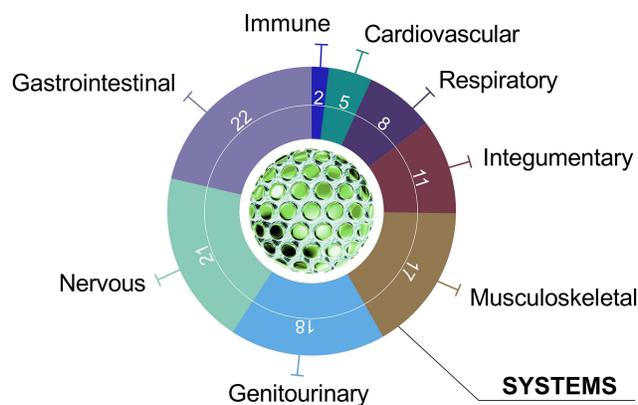
**Table 3** Overview of the Analytical Methods Used for the Characterization of Phytosomes Featured in This Review

Parameter	Techniques
Size, and shape	DLS, SEM, TEM, Optical microscopy, Fluorescence microscopy, AFM, Field flow fractionation, Nanoparticle tracking analysis, Scanning ion occlusion sensing, Flow Cytometry, Size-exclusion chromatography, Centrifugal sedimentation, and DSC.
Surface charge	DLS, free-flow electrophoresis, and laser Doppler velocimetry.
Chemical composition	FTIR, $^1\text{H}$ NMR, GC-MS, LC-MS, DSC, TGA, and Thin-layer chromatography.
Lamellarity and stability	$^{31}\text{P}$ nuclear magnetic resonance, Small-angle X-ray scattering, electron microscopy methods, DSC, TGA, DLS, and UV-Vis.
Encapsulation Efficiency and release behavior	Mini-column centrifugation, HPLC, UPLC, UV-Vis, dialysis, enzymatic assays, gel electrophoresis, field flow fractionation, sample-and-separate approach, the in-situ method, and the continuous flow.
Optimization	Design of Experiment (DoE) with Box–Behnken design

**Abbreviations:** DLS, dynamic light scattering; SEM, scanning electron microscope; TEM, transmission electron microscopy; AFM, atomic force microscope; DSC, differential scanning calorimetry; FTIR, Fourier-transform infrared spectroscopy; NMR, nuclear magnetic resonance; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; TGA, thermal gravimetric analysis; HPLC, high-performance liquid chromatography; UPLC, ultra-performance liquid chromatography.

## Biological Activities of Phytosomes

The biological activities related to phytosomes are heterogeneous and have been evaluated in more than 100 studies. To simplify the analysis of the results, papers were divided based on the body district involved. Accordingly, the phytosome effects on the following categories have been investigated: cardiovascular, central and peripheral nervous, gastrointestinal, genitourinary, immune, integumentary, musculoskeletal, and respiratory systems. Finally, the last paragraph was devoted to the effects of phytosomes in metabolic syndromes. Figure 6 reports the number of papers related to phytosome and their biological activities, divided according to the system under study, whereas Figure 7 collects the number of studies on phytosome based on the main natural constituent.

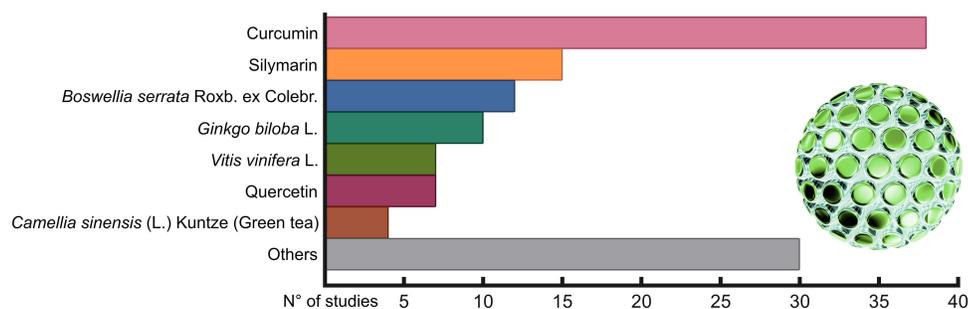


**Figure 6** Biological activities of phytosomes by system. The graph shows the number of papers related to phytosomes and their biological activities, divided according to the system under study. The gastrointestinal, nervous, genitourinary, and musculoskeletal systems together account for almost the 75% of the published works. Systems involved in metabolic syndrome were not considered.

## Phytosomes and Cardiovascular Protection

The isoproterenol (ISO)-induced cardiotoxicity model has been used to evaluate the protective effects of *Ginkgo biloba* phytosomes in rats. The results showed that *Ginkgo biloba* phytosome (200 mg/kg) alleviated ISO-induced myocardial necrosis considerably, as confirmed by histopathological studies. Moreover, the myocardial necrosis diminished and the endogenous antioxidants were increased, thus overall making evident the cardioprotective effect.<sup>171</sup> The same researchers explored the possible protection by cardiovascular injuries of a combined treatment of *Ginkgo biloba* phytosome (100 mg/kg) and *Ocimum sanctum* extract (OS) (50 and 75 mg/kg) in isoproterenol (ISO) (85 mg/kg)-induced myocardial necrosis in rats. The treatment inhibited the increase of serum marker enzymes and the lipid peroxidation marker malondialdehyde (MDA), both induced by ISO. However, none of the combined treatments possessed better cardioprotective or antioxidant activity than the single treatment with *Ginkgo biloba* phytosome or OS.<sup>172</sup>

Tisato et al investigated the anti-inflammatory effect of *Ginkgo biloba* phytosome and  $\alpha$ -Lipoic acid on cytokines and chemokines released by vein endothelial cells (VEC) isolated from patients at different stages of CVD. The anti-inflammatory effects of both *Ginkgo biloba* derivatives and  $\alpha$ -Lipoic acid were confirmed by the reduction of cell adhesion molecules ICAM-1 and VCAM-1. *Ginkgo biloba* phytosome diminished the basal release of PDGF and the TNF- $\alpha$ -induced PDGF, CXCL10, and RANTES levels. Based on the data collected,  $\alpha$ -Lipoic acid exhibited a wider and more potent inhibitory activity on the release of cytokines/chemokines concerning *Ginkgo biloba* phytosome. This study recognized that  $\alpha$ -Lipoic acid markedly



**Figure 7** Main natural products in phytosomes. The graph collects the number of studies on phytosomes based on the main natural constituent. The columns group botanical or active principle with 3 or more publications, while constituents with 2 or less studies have been collected in "Others". The data show a higher prevalence of phytosomes loaded with pure compounds rather than natural extracts, especially curcumin. All the references corresponding to the individual studies considered are reported along the manuscript.

counteracted TNF- $\alpha$ -induced NF- $\kappa$ B and p38/MAPK activation, whereas *Ginkgo biloba* mostly acted on Akt.<sup>173</sup>

A commercial formulation was examined in a large sample of CVD patients enrolled in 54 Italian centers. The supplement contains phytosome of polyphenolic extract from *Vitis vinifera* L. seeds, extract from *Melilotus officinalis* (L.) Pall, and bromelain 100 mg. A total of 648 patients were enrolled and received 1 tablet/day and/or standard compression stockings for 90 days. In all groups, it was reported a notable reduction in the malleolus circumference, both at the left and the right limb. A comparable pattern was observed for the severity of the disease and symptomatology.<sup>174</sup>

Muir et al have investigated the clinical efficacy of *Ginkgo biloba* phytosome, in the treatment of Raynaud's phenomenon (RP). A painful condition characterized by episodic digital ischemia. A total of 22 patients with RP and without other associated conditions were enrolled. A number of 11 patients were randomized to receive *Ginkgo biloba* extract (120 mg three times a day for a final amount of 360 mg/day), while 11 patients received matching placebo. The number of RP episodes per week before treatment with *Ginkgo biloba* phytosome ( $13.2 \pm 16.5$ ) was reduced by 56%, whereas the placebo reduced the number by only 27% ( $p < 0.00001$ ). There were no significant dissimilarities in hemorheology among the two groups.<sup>175</sup>

## Evidence on the Role of Phytosomes in the Nervous System

### The Phytosomes in Cognitive Impairment and Neuronal Damage

Several papers report the bioavailability of phytosome concerning the corresponding unformulated products in animal models, focusing on the tissue distribution of the active ingredients. Husch et al found a greater amount of Boswellia acids from *Boswellia serrata* (ie, KBA, AKBA,  $\beta$ BA) following the administration of Boswellia-loaded phytosome.<sup>176</sup> Another study investigated phytosome formulation loaded with *Annona muricata* water extract intending to ameliorate its permeability across the blood-brain barrier (BBB), thus improving the antidepressant-like activity due to inhibition of monoamine oxidase B (MAO-B). Through an in vitro transwell model of BBB, phytosome formulation registered the best performance as a radical scavenger and MAO inhibitor, thus representing a useful model to improve the antidepressant-like activity of the extract.<sup>177</sup> La Grange et al investigated the ability of

silymarin phytosome to protect fetal rat brain by ethanol administration. Silymarin is a complex of flavonolignans from *Silybum marianum* Gaertn., namely milk thistle. The activity of antioxidant enzymes, which include gamma-glutamyl transpeptidase, was generally higher in the group treated with the phytosome formulation.<sup>178</sup>

Two different studies have been carried on by Naik et al on the biological activities of a *Ginkgo biloba* phytosome formulation in Wistar rats; in the first study, oral administration at 50 or 100 mg/kg, reduced pentobarbitone-induced sleeping time, decreased the chlorpromazine effects, and induced spontaneous motility in rodents. Moreover, the formulation exhibited antidepressant effects in the amnesia induced by scopolamine, showing general improvement in the behavioral tests.<sup>179</sup> The second study evaluated the antioxidant activity in the rat brain after acute (7 days) or subchronic treatment (14 days). Brain areas including the cerebellum, striatum, cerebral cortex, and hippocampus were isolated, and the activity of the antioxidant enzymes, GPx, SOD, CAT, and GR, was tested, finding phytosomes-induced increased activities in the brain areas analyzed.<sup>180</sup>

Ullah et al studied the ability of a curcumin phytosome to decrease glial activation in GFAPIL6 mice, an animal model of chronic glial activation. Formulation administered at three doses (218, 438, and 874 ppm) for four weeks caused a decrease of neuroinflammation and number of activated microglia in the hippocampus ( $-26.2\%$ ) and the cerebellum ( $-48\%$ ).<sup>181</sup>

Recently, our group demonstrated that *Centella asiatica* phytosome administered to adult male rats for ten days at 20 and 100 mg/kg (calculated as triterpene equivalents) induced BDNF increase in the prefrontal cortex, and the higher dose generally counteracted cognitive impairment. In the NOR test, the increase in the preference index was accompanied by increased levels of the Bdnf expression. In addition, there were no side effects observed during the treatment.<sup>182</sup> In another paper, we demonstrated that phytosome loaded with *Centella asiatica* and *Curcuma longa* extracts, administered chronically to rats (50 or 250 mg/kg for ten days), affected local protein synthesis through the modulation of BDNF-mTOR-S6 pathway. Our findings supported the use of this preparation in subjects with memory and cognitive impairment.<sup>101</sup>

### The Phytosomes in Neurodegenerative Diseases

Neurodegenerative brain dysfunction is responsible for the development of dementia in aged people. Bahadur S.

investigated the nanoparticle system to improve the drug delivery or active compounds with poor availability to the brain.<sup>183</sup> Langasco et al studied the brain delivery of the isoflavone genistein testing various nanotechnological approaches; oxidative stress in PC12 cells (neuron cell line) was diminished by treatment with phytosomes, and the effect was better than the unformulated genistein.<sup>184</sup> Among phytochemicals, curcumin phytosome was found to increase curcumin bioavailability in the hippocampus and frontal lobe following repeated oral administration of the formulation for five days (134 mg/kg/die as curcuminoids equivalent) in rats. In the frontal lobe, curcumin appeared 30 minutes after treatment, peaked at 1 hour, and tended towards normalization after 3 hours, demonstrating that curcumin phytosome can reach the brain in rats.<sup>185</sup> Since curcumin possesses anti-amyloid and anti-inflammatory activities, which are mostly used against neurodegenerative diseases including Alzheimer's disease, this finding may be useful for future studies aimed at better design drug delivery.<sup>186</sup>

### The Phytosomes in Cerebral Ischemia

Two studies from the same group investigated the potential positive effects of natural compounds in the middle cerebral artery occlusion model in rats. Rutin, a glycoside of the flavonoid quercetin, has been loaded in a phospholipid structure and tested for its bioavailability in an animal model of cerebral ischemia. LC-MS/MS analysis revealed that rutin, administered at 100 mg/kg to Sprague Dawley rats, reached the brain at concentrations ranging from 20 to 50 ng/g. Rutin-loaded formulation highly ameliorated functional outcomes in an animal model of stroke.<sup>187</sup> In the second study, a phytosomal complex containing the ethanolic extract of Ashwagandha (*Withania somnifera*) roots was administered orally (85 mg/kg) to rats 1 hour before ischemia and six hours post-reperfusion. Treatment provoked a strong reduction of cerebral infarction (82.7%) and afforded better protection on all neurological deficit parameters.<sup>188</sup>

### Effect of Phytosomes in Neuropathy

Di Pajardi et al studied the clinical potential of oral treatment (3 months, n=180) of, curcumin phytosome (500 mg),  $\alpha$ -lipoic acid (300 mg), and vitamins of the B group in subjects with carpal tunnel syndrome awaiting surgical treatment. Patients receiving supplementation for three months twice/day both before and after surgery showed a decrease of night symptoms at 40 days after surgery and

were less likely to reach a positive Phalen's test at 3 months post-surgery.<sup>189</sup> In neuropathic patients, a similar formulation based on curcumin phytosome and piperine and/or  $\alpha$ -lipoic acid reduced pain (-66%) in all the combinations, after 8 weeks. The supplementation decreased by 40% the use of conventional therapy (ie, dexibuprofen), whereas lipoic acid alone did not show statistically significant results.<sup>190</sup>

### The Phytosomes in Migraine

In two studies of the same research group, the efficacy of *Ginkgo biloba* terpenes phytosome (60 mg), vitamin B2 (8.7 mg), and coenzyme Q10 (11 mg) as ingredients, administered twice daily, was investigated in fifty subjects suffering from migraine with aura. Positive effects in reducing migraine with aura, both frequency and duration, were already clear within a four-month treatment. These effects were probably due to the presence of ginkgolide B, the most abundant terpene identified in the *Ginkgo biloba* leaf extract.<sup>191</sup> Ginkgolide B was found to modulate/reduce the glutamate neurotransmission in the CNS, which plays a pivotal role in the onset of migraine.<sup>192</sup> The efficacy of the same formulation in the acute stage of migraine with aura was tested in an open study; during the first symptoms of aura, patients orally consumed two capsules of *Ginkgo biloba* terpenes phytosome, with no restriction on analgesic intake during the pain phase. About 60% of patients enrolled in the study experienced a reduction of neurological symptoms after treatment; moreover, the pain phase was completely abolished in almost 20% of patients.<sup>193</sup>

Balzano et al investigated the beneficial effects of a mixture of magnesium, vitamins (riboflavin, niacin, vitamin D), L-tryptophan, and the *Boswellia serrata* extract-loaded phytosome, in patients with transient tension migraine and migraine without aura. The authors considered pain modulation (NRS scale), monthly attack number, and analgesic intake. Amitriptyline was used as a reference compound. The authors found an improvement in all the outcomes, with greater compliance and no side effects for patients who consumed the phytosome formulation.<sup>194</sup>

### The Phytosomes in Nervous System Cancer

Glioblastomas are among the most aggressive malignancies affecting the central nervous system. To search for novel strategies to cope with the disease, Mukherjee et al studied the ability of the intranasal delivery of curcumin phytosome (500 mg, corresponding to 96 mg

curcuminoids) to cause remission of glioblastoma in the brain of GL261 (glioblastoma cells)-implanted mice. Tumor remission was observed in 50% of mice; similar effects were achieved also using intraperitoneal infusion. Therefore, the authors suggest that curcumin-loaded phytosome could affect the viability of glioblastoma cells and also induce repolarization of microglia cells to the tumoricidal M1 state.<sup>195</sup> Similar results were obtained by the same group studying the effects of curcumin phytosome in natural killer cells and macrophages in GL261 (glioblastoma cells)-implanted mice. The treatment also induced suppression of proteins STAT3 and ARG1, and IL-10 induction of STAT1; suppression of inducible nitric oxide synthase and caspase 3 activation in the glioblastoma cells were also observed.<sup>196,197</sup>

The same curcumin phytosome was investigated in an animal model (D425MED) of medulloblastoma, the most common pediatric central nervous system cancer. The results reveal negligible effects of formulation using either oral or intraperitoneal administration; however, no information on the dose used was reported.<sup>198</sup>

Di Pierro et al studied the efficacy of a *Boswellia* extract as a phytosome in cerebral edema induced by radio-chemotherapy in patients with glioblastoma. Patients (n=20) received temozolomide and 4500 mg/die of formulation for a maximum of 34 weeks. The stage of the disease was evaluated at different times ranging from 4 to 34 weeks post-surgery, together with steroid consumption. Two subjects showed a significant decrease in brain edema, thus leading to better surgical resection. The authors conclude that supplementation with this type of phytosome may elicit positive effects in reducing cerebral edema induced by radio-chemotherapy, and the brain edema reduction may decrease dexamethasone intake, thus minimizing steroid-induced side effects during conventional pharmacological treatment.<sup>199</sup>

## The Phytosomes in the Gastrointestinal System

### The Phytosomes and Gut Microbiota

A recent study compared the influence of the two different curcumin-based products, unformulated curcuminoids, and lecithin-curcuminoid formulation, on human colonic metabolism. Both extracts were subjected to fermentation using an in vitro fecal model mass spectrometry was used for curcuminoid quantification and assessment of possible curcuminoid degradation and detection of the main metabolites in the human fecal fermentation. The results

showed that the fermentation of lecithin-formulated curcuminoids caused a more pronounced occurrence of curcuminoid catabolites.<sup>200</sup>

### The Phytosomes and Pancreatic Cancer

The potential synergistic effects of gemcitabine and the curcumin phytosome in advanced pancreatic cancer were evaluated in a prospective Phase II trial. A total of 44 patients affected by locally advanced or metastatic pancreatic cancer were enrolled and received 2000 mg/die daily (4 capsules, each of 500 mg) in addition to gemcitabine (10 mg/m<sup>2</sup>/min, infusion over 100 min on days 1, 8, 15 every 28 days). The response rate was the primary endpoint of this study; progression-free survival, overall survival, quality of life, and tolerability were the secondary endpoints. The results of the study suggest that curcumin phytosome can be used as a complementary treatment associated with gemcitabine in the therapy of pancreatic cancer.<sup>201–203</sup>

### The Phytosomes Against Bowel Inflammation

An open-label, observational, registry study estimated the effects of a lecithin-based delivery form of standardized *Boswellia serrata* extract in patients with minimally symptomatic ulcerative colitis in the remission phase. The 43 patients freely decided to receive 1 tablet of 250 mg/day or no supplementation for 4 weeks. Diffuse intestinal pain, bowel movements and cramps, watery stools, blood in stools, anemia, malaise, rectal involvement, and the number of white blood cells were attenuated in the supplementation group. The need for other drugs and medical examinations was also reduced.<sup>204</sup>

Two clinical studies evaluated the efficacy and safety of *Boswellia serrata* extract phytosome in irritable bowel syndrome (IBS). In the first, 71 healthy subjects with idiopathic IBS were assigned in three groups and received hyoscine butyl bromide, papaverine hydrochloride + *Atropa belladonna* extract, both administered when needed, or 1 tablet of phytosome (250 mg/day) for 4 weeks. IBS symptoms showed improvements in all groups, but only in the phytosome consumption group a substantial decrease in the need for medical attention and a lower occurrence of side effects, mainly stypsis, was detected.<sup>205</sup> The second perspective, a controlled, randomized study evaluated the long-term efficacy and the safety of phytosome for the prevention of symptoms in healthy subjects with mild IBS. The same management strategies of the previous study were applied to 71

subjects. At the follow-up (6 months), compared to the groups receiving the standard treatment, the phytosome group exhibited a lower mean score value for nearly all self-assessed IBS symptoms and a considerably lower need for medicines and consultations or medical evaluation/admissions.<sup>206</sup>

### Efficacy of the Phytosomes in Bowel Cancer

The beneficial effects of oral silibinin and silybin-phytosome against human colorectal HT29 xenograft growth were compared in vivo in athymic nude mice. A dosage of 100 mg/kg of the silybin-phytosome exhibited an efficacy close to silibinin 200 mg/kg in reducing tumor weight and volume.<sup>207</sup>

Efficacy of the combination of oxaliplatin and curcumin phytosome was investigated in vitro, in oxaliplatin-resistant cells, and in vivo, in colorectal tumor-bearing mice. This combination, compared with oxaliplatin alone and control, improved the antiproliferative capacity of oxaliplatin in vitro. A positive effect was observed also in the HCT116 nude mouse xenograft model, with a decrease of tumor volume, a decrease of pharmacodynamic markers Ki-67 and Notch-1, and an increase of cleaved caspase-3.<sup>208</sup> Another study investigated the efficacy of phytosomal curcumin or in association with 5-fluorouracil (5-FU) in vitro or in vivo model of colon tumor with the presence of colitis. In CT26 cells, curcumin inhibited cell growth in a concentration-dependent fashion (0–1000 µg/mL) and notably improved the expression of E-cadherin. A combination of curcumin (25 mg/kg/day) + 5-FU (35 mg/kg/weekly) diminished the tumor-number and tumor-size in mice for curcumin or 5-FU alone.<sup>209</sup> The same combination of phytosomal curcumin and 5-FU was used in a xenograft mouse model of colorectal cancer. The study showed tumor growth reduction, an increase in the antitumor effect of 5-FU, and anti-angiogenic effects across modulation of VEGF and VEGFR2.<sup>210</sup>

### Hepatoprotective Effects of the Phytosomes

La Grange et al investigated the efficacy of silymarin-phytosome in the protection of the fetus from maternal ethanol ingestion in rats. It was compared to the activity of oral with subcutaneously injected phytosome, with doses ranging from 400 to 800 mg/kg. All doses suppressed gamma-glutamyl transpeptidase (GGTP) activity induced by ethanol in brain and liver tissue, in both the fetuses and the dams. The highest dose of phytosome administered orally appeared optimal in reducing maternal brain and

fetal GGTP activity. According to the authors, there may be a protective activity of the formulation of ethanol toxicity, as well as direct inhibition of GGTP without protective activity.<sup>178</sup>

The hepatoprotective effects of *Ginkgo biloba* phytosome on carbon tetrachloride (CCl<sub>4</sub>)-induced hepatotoxicity were investigated in rats. *Ginkgo biloba* phytosome was administered for 10 days in two doses, 25 mg/kg and 50 mg/kg i.p., and silymarin (200 mg/kg P.O.) was used as the standard reference. Phytosome decreased enzyme levels of glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and alkaline phosphatase (ALP) in serum; levels of SOD, CAT, GPx, GR, albumin, and total proteins were significantly increased, and the GSH levels were found close to control levels. On some parameters, the effect of the higher dose of *Ginkgo biloba* phytosome was comparable with silymarin.<sup>211,212</sup> The same group investigated the hepatoprotective effects of phytosome on rimfacin-induced hepatotoxicity in rats. Also in this study, *Ginkgo biloba* phytosome was administered at 25 mg/kg and 50 mg/kg, showing hepatoprotective effects by reducing the levels of serum marker enzymes and lipid peroxidation; treatment increased the levels of SOD, GSH, GPx, GR, CAT, albumin, and total protein in a dose-dependent manner.<sup>213</sup>

A Phase III, double-blind, placebo-controlled, randomized clinical trial evaluated the beneficial activities of silybin combined with vitamin E and phosphatidylcholine on liver function in patients with non-alcoholic fatty liver disease (NAFLD). Several 180 patients with NAFLD (36 with HCV chronic infection) were enrolled to receive orally active treatment (n=91) (silybin 94 mg, phosphatidylcholine 194 mg, vitamin E acetate 50% 89.28 mg, twice daily) or placebo (n=88) for 12 months. In patients receiving the active treatment, improvements in insulin resistance, transaminases and  $\gamma$ -glutamyltransferase ( $\gamma$ GT) levels and several aspects of liver histology were observed. In patients HCV-positive, the active treatment improved markers of fibrogenesis.<sup>214</sup>

Ali et al examined the effects of silybin phytosome (400 mg/kg), curcumin (400 mg/kg), or  $\alpha$ -R-lipoic acid (200 mg/kg), all given orally, in a model of thioacetamide-induced liver cirrhosis in rats. All supplements significantly decreased serum levels of GPT, GOT, LDH, and  $\gamma$ GT; only serum ALP levels were not decreased by silybin phytosome. Collagen deposition, matrix metalloproteinase (MMP)-2 activity (MMP-2), TGF- $\beta$ 1 level, and HSP-47 gene expressions were also reduced. Moreover, all

supplements improved the oxidative stress status through the increase of liver GSH and the reduction of MDA levels.<sup>215</sup>

Another study compared hepatoprotective activities of silymarin phytosomes and milk thistle extract (both given orally as 200 mg/kg/day silybin equivalent for 10 days) in CCl<sub>4</sub>-induced hepatotoxicity in rats. Silymarin phytosome increased SOD and decreased GPT levels more efficiently than milk thistle extract ( $p < 0.05$ ). No significant difference between the two treatments regarding other biochemical parameters was observed.<sup>216</sup>

The bioavailability of a standardized pomegranate extract (30% w/w punicalagin – SPE) and soy phospholipids was compared with unformulated SPE in rats treated with CCl<sub>4</sub>. Pharmacokinetic studies showed that the formulation of pomegranate extract and soy phospholipids (500 mg/kg equivalent SPE) led to the serum concentration of punicalagin higher than SPE ( $C_{max}$  466.3 ng/mL and 192.5 ng/mL respectively). Antioxidant activity was evaluated at two doses (100 and 200 mg/kg) as well. Compared with SPE, pomegranate extract and soy phospholipid combination significantly preserved the concentrations of the liver enzymes SOD, glutathione system, CAT.<sup>217</sup>

In vivo *Boswellia serrata* extract phytosome significantly decreased the serum levels of the pro-inflammatory cytokines TNF- $\alpha$  and IL-6 and increased the levels of the anti-inflammatory cytokine IL-10 in lipopolysaccharide-induced systemic inflammation in mice. Phytosome showed antioxidant capacities through a significant attenuation of lipid peroxides and increased levels of GSH, glutathione disulfide, and total glutathione concentrations. Moreover, treatment was able to restore CYP transformation and consequently re-establish the biotransformation capacity in the liver.<sup>218</sup>

The hepatoprotective activity of a phytosome formulated with the combination of dry ethanolic extracts from *Piper longum* fruits and *Abutilon indicum* leaves was compared with dry ethanolic extracts from each plant alone and with LIV 52, an Ayurvedic formulation indicated for liver disorders. Phytosome (100 mg/kg), dry ethanolic extracts (100, 200, 400 mg/kg), and LIV 52 (1 mL/kg) were administered orally to rats with liver damage induced by CCl<sub>4</sub>. Phytosome reduced liver damage markers (GPT, GOT, ALP, and bilirubin) to a greater extent than dry ethanolic extracts and in a similar manner to LIV 52.<sup>219</sup>

Hepatoprotective effects of curcumin phytosome were investigated in a model of aluminum chloride (AlCl<sub>3</sub>)

induced hepatotoxicity in rats. Compared with the untreated AlCl<sub>3</sub> group, treatment with phytosome (200 mg/kg/day for 21 days) notably normalized the hepatic markers increased by AlCl<sub>3</sub> (GOT, GPT, ALP, LDH, and bilirubin).<sup>220</sup>

The chemopreventive effect of curcumin phytosome was evaluated and compared with unformulated curcumin on hepatitis B virus related-hepatocellular carcinoma by using a transgenic mouse model. Phytosome showed greater efficacy in reducing hepatocellular carcinoma growth, reduction of total tumor volume, and anti-inflammatory activity than unformulated curcumin.<sup>221</sup>

## The Phytosomes Effect in the Genitourinary System

This section describes the biological activities, which affect the genitourinary tract, including the breast, as a gland linked to the reproductive system.

### The Phytosomes and Breast Cancer

In the first study, twelve early breast cancer patients were treated for 4 weeks with a commercial lecithin formulation containing catechins from green tea, at a daily dose of 300 mg (corresponding to 44.9 mg of epigallocatechin-3-gallate or EGCG) before surgery. The research showed the ability of the active principles to reach human breast tissue; concentrations up to 8 ng/g of EGCG were found in all the tumor tissues tested. The evaluation of Ki-67, as a biomarker of proliferation, demonstrated a significant inverse correlation with EGCG plasma levels for each patient.<sup>222</sup>

The same research group evaluated the activity of a complex of silybin-phosphatidylcholine, in another group of 12 breast cancer patients, 2.8 g per day for 28 days. The concentration of silybin reached up to 177 ng/g in breast tumor tissues, but non-changes in Ki-67 was noted, as well for insulin-like growth factor 1 (IGF-I) and nitric oxide blood levels.<sup>223</sup> In vitro, silybin-phosphatidylcholine treatments obtained a concentration- and time-dependent decrease in viability of SKBR3, a cell line of human breast adenocarcinoma, confirming a superior membrane transmission (more than 1.5 times) and inhibitory effect on growth (more than 2 times) compared to pure silybin. Both silybin and silybin-phosphatidylcholine downregulated Human Epidermal Growth Factor Receptor 2 (HER2) expression, but the complex gave better results in longer treatment times (72 h).<sup>224</sup> A study prepared and evaluated the effect of phytosomes containing luteolin in

MDA-MB 231 breast tumor cells. Phytosomes increased the activity of doxorubicin in inhibiting the growth of cancer cells, compared to the pure compound. Besides, the related phytosomes were more active than luteolin in inhibiting the gene expression of nuclear factor erythroid 2-related factor 2 (Nrf2), lowering the antioxidant defense of cancer cells.<sup>225</sup>

Similarly, quercetin phytosomes also increased the efficiency of doxorubicin on the growth of MCF-7 human breast cancer cells. Despite no significant effects were observed on Nrf2 gene expression, quercetin phytosomes decreased the expression of two Nrf2-activated genes, NAD(P)H dehydrogenase (quinone) 1 (35%) and multidrug resistance-associated protein 1 (43%), more efficiently than pure quercetin.<sup>226,227</sup>

MCF-7 cells treated with a commercial phytosomal-curcumin, showed a dose–response inhibition of proliferation and invasion, linked to higher levels of E-cadherin and MMP-9. Moreover, phytosomal-curcumin enhanced the biological activity of fluorouracil in inhibiting tumor growth of a xenograft mouse model (female BALB/c), by positively regulating MDA levels, catalase, total thiol concentration, and SOD in breast cancer tissue. However, the phytosome alone, without fluorouracil, reduced growth to a lesser extent, without modulating the individual parameters.<sup>228,229</sup>

Phytosomal-curcumin was tested in female BALB/c mice with metastatic breast tumor (4T1). Mice were fed with the phytosomal-curcumin for 14 days with 10 mg once every 3 days. While the treatment alone had minor effects on the primary tumors, it significantly decreased the number of metastases in the lung at a dose of 10 mg/mouse. Although this study lacks comparative data on pure curcumin, the animals treated with cryoablation and phytosome did not improve their survival rate with respect to the animals with saline, or just cryoablation or phytosomal-curcumin alone.<sup>230</sup>

Finally, two studies by the same research group evaluated phytosome bilayer-enveloped casein micelles or phosphatidylcholine-casein micelles, containing *Monascus* yellow pigments (*Monascus purpureus*) and resveratrol, by comparing also folate conjugated and PEGylated phytosome modifications. All forms of phytosomes induced higher toxicity in MCF-7 cells comparing to the cotreatment of free resveratrol and *Monascus* yellow pigments. Tumor-bearing BALB/c mice received through injection the pure compounds/mixtures or phytosomes, corresponding to 5 mg/kg per day of resveratrol, for consecutive 21 days. At 250 µg/mL, the percentage of hemolysis induced by phytosomes was lower than 5%. Phytosomes were superior in tumor regression

concerning coadministration of free resveratrol and *Monascus* yellow pigments. Treatments with phytosomes better-reduced aromatase, NF-κB, VEGF, and CD1 levels, and increased caspase-3 level and necrosis.<sup>231,232</sup>

### The Phytosomes Role in Prostate Diseases

Three studies evaluated the effects of silibinin-loaded phytosome, in the field of prostate cancer. In the first in vivo study, TRAMP male mice, characterized by a palpable prostate tumor, were exposed to 0.5% or 1% w/w of a phytosome diet. After 11 weeks, the diet dose-dependently decreased the weight of the prostate together with tumors (up to 60%), suppressing metastasis formation by reducing fibroblast growth factor (bFGF), VEGF, MMP-2, and MMP-3. Silibinin led to higher levels of E-cadherin in parallel with a reduction of vimentin and also snail-1 in tumors.<sup>233</sup> Two clinical studies evaluated the effects on humans. The first pharmacokinetic Phase I study involved 13 subjects with prostate cancer. Phytosome was increased from a 2.5 g to a 20 g orally daily dose, but a persistent grade 2 hyperbilirubinemia was registered at 15 and 20 g. Silibinin, rapidly conjugated, was released into the urine, pointing out a short plasma half-life, in a range of 1.79–4.99 h. None of the patients under study achieved a 50% reduction in PSA, but several patients experienced a prolonged stable disease.<sup>234</sup>

The second study from the same research group administered 13 g of silibinin phytosome daily to 6 prostate cancer patients, for 14–31 days before radical prostatectomy. The plasma silibinin levels were relatively low (1.2 µM) at the end of the treatment. Only three patients out of six showed silibinin values reaching from 14.9 to 496.6 pmol/g in prostatic tissues. IGF-I, IGFBP-3, or PSA levels were not significantly changed.<sup>235</sup>

The latest research investigated the application of curcumin phytosome formulation in patients with benign prostatic hyperplasia. The phytosome was administered as two tablets per day (2 × 500 mg per day, equivalent to 200 mg of daily curcumin) to 33 subjects (range: 55–65 years) in association with the best standard management. All symptoms including urination frequency, intermittency, urgency, straining, and nocturia improved with curcumin administration compared to standard management, except for stream weakness.<sup>236</sup>

### The Phytosomes in Female Reproductive System Conditions

A clinical study evaluated the effect of curcumin phytosome in 6 patients with endometrial cancer. Patients

received the supplement for 2 weeks with 2 g ( $4 \times 500$  mg per day) without simultaneous oncological treatments. Supplementation lowered MHC expression on leukocytes, the number of monocytes, and ICOS protein levels on CD8 +T cells. No other significant changes were observed in inflammatory markers, such as number of different immune cell types, activation of T cell, and the protein levels of cyclooxygenase-2 (COX-2).<sup>237–239</sup>

A second study evaluated the effect of a quercetin phytosome (10 or 50 mg/kg, per os) in 48 rats subjected to ovariectomy. Treatment with phytosome induced a significant increase of calcium, inorganic phosphorus, and glutathione in serum, compared to the corresponding doses of free quercetin. Compared to quercetin, the phytosome significantly lowered serum alkaline phosphatase, TNF- $\alpha$ , acid phosphatase, MDA, and glucose level and also positively modified the lipid profile.<sup>131</sup> Furthermore, a study evaluated an icariin-containing phytosome in OVCAR-3 ovarian cancer cells. The phytosomes showed higher cytotoxicity versus ovarian cancer cells compared to pure icariin (6.31 vs 13.1  $\mu$ M) and in particular, the number of cells in the G2-M phase, the caspase-3 content, and intracellular ROS were enhanced following incubation with phytosomes.<sup>240</sup>

### The Phytosomes in Urinary Tract Dysfunctions

Two clinical trials evaluated the biological effects of phytosomes in the urinary tract. In the first research, cranberry was studied in 13 healthy volunteers to evaluate the *Candida albicans* antiadhesive properties of urine after cranberry extract phytosome or the corresponding standardized extract consumption. The subjects consumed 2 capsules of cranberry phytosome or cranberry extract per day, for a week and urines were analyzed at different times. The fractions retrieved after 12 h of extract or phytosomal form treatment significantly and similarly inhibited the adhesion of *C. albicans*, but phytosome contained only 33% of the cranberry extract (phytosome: 12 mg proanthocyanidins/capsule; extract: 36 mg proanthocyanidins/capsule).<sup>241</sup>

The second study explored the effect of curcumin in asymptomatic patients suffering from temporary kidney dysfunction. Patients consumed 3 capsules/day for 4 weeks, containing curcumin phytosome (300 mg of curcumin). The subjects treated with curcumin phytosomes had significantly higher improvement in micro- and macroalbuminuria and oxidative stress levels than those on standard management. The number of patients experiencing

fatigue was significantly reduced by curcumin phytosome, and compliance and tolerability were good.<sup>242</sup>

## The Phytosomes as Modulators of the Immune System

A couple of studies evaluated the effects of phytosome on parameters related to immune function. Silymarin loaded on liposome/phytosome (lecithin: cholesterol ratio 6:1) showed improved prevention of ROS release compared to unformulated silymarin in RAW 264.7, a murine macrophage cell line. In vivo study performed for seven days in Wistar rats (50 mg/kg) exerted protection against liver toxicity and inflammation induced by paracetamol.<sup>243</sup> Another study evaluated the immunomodulatory effects of the phytosomal formulation of grape seed extract, that is particularly rich in epigallocatechin 3-O-gallate. One-month administration of grape seed phytosome (300 mg/die) to elderly patients influenced the immune response, as shown by serum cytokine assessment. In particular, the treatment increased both IL-2 and INF $\gamma$  production, thus suggesting a possible role in the Th1/Th2 rebalance in atopic frail elderly or the enhancement of antiviral response.<sup>244</sup>

## The Phytosomes Effect in Integumentary System

The formulations evaluated at the skin level are more disparate and can be collected in three main areas: skin inflammatory conditions, wound healing, and skin cancer.

### The Phytosomes in Skin Inflammatory Conditions

Two clinical studies showed the effect of phytosomes in the field of skin inflammation. A first blind trial with 30 volunteers investigated the topical effect of a quercetin phytosome, in comparison to a formulation containing 1% dexchlorpheniramine, in different types of skin insults. Quercetin phospholipids 1% and dexchlorpheniramine 1% obtained similar results by significantly reducing UV-induced erythema ( $-10.05\%$  vs  $-14.05\%$ , respectively) and in histamine prick test ( $-13.25\%$  vs  $-12.23\%$ , respectively). When erythema was induced by sodium lauryl sulfate (SLS) or glycolic acid (GA) only quercetin phospholipids 1% induced a significant increase in hydration, but both the formulations reduced erythema.<sup>245</sup> In a Phase III randomized, single-dose, and double-blind placebo clinical trial, 49 patients with chronic psoriasis were treated orally for 12 weeks with phytosome (2 g per day) or placebo, while topically applying once daily methylprednisolone acetate 0.1% ointment on psoriasis plaques. Curcumin

phytosome obtained a better effect on PASI compared to placebo. No significant reduction of IL-17 serum levels was observed between the groups, but IL-22 serum levels were lower in the curcumin treated group ( $-11.8$  pg/mL).<sup>246</sup> Another study evaluated the effects of curcumin phytosomes in carrageenan-treated mice. Indomethacin, curcumin, or nano-phytosome of curcumin at 15 mg/kg were administered orally to animals for one week. The nano-phytosome treatment was more antioxidant than curcumin ( $P < 0.05$ ) in the case of SOD, CAT, GPx, and GR and had a higher latency time compared to curcumin in hot plate and tail-pinch tests.<sup>247</sup>

Three studies evaluated the topical effects of three different phytosomes in carrageenan-induced edema in Wistar rats. The Lawsonia-containing phytosome complex (*Lawsonia inermis* L.) had a higher anti-inflammatory effect than plant extract gel at 4 h ( $P < 0.001$ ).<sup>30</sup> Escin  $\beta$ -sitosterol (ES) phytosome 5% hydrogel showed significantly improved efficacy in antihyperalgesic activity compared to escin and ibuprofen 5% gel.<sup>248</sup> A resveratrol phospholipid complex, topically applied with patches, reduced the swelling to 6.1% after 24 h, a value significantly lower than control (38.4%) and diclofenac sodium gel groups (23.2%) ( $P < 0.05$ ). Resveratrol phospholipid complex containing patches resulted in non-irritant effects in albino rabbits, with a skin irritation score (erythema and edema) of less than 1.<sup>249</sup> Silymarin in nanostructured lipid carriers (NLC) complex was topically applied in rats subjected to UVB irradiation ( $0.115$ – $0.23$  J/cm<sup>2</sup>). Silymarin-NLC gel application decreased the epidermal thickness and wrinkle score in UV exposed animals.<sup>250</sup>

### The Phytosomes Effect in Wound Healing

A combination of *Ginkgo biloba*,  $\alpha$ -lipoic acid, and grape seed phytosome associated with advanced medications, was beneficial in the treatment of chronic diabetic ulcers in subjects with diabetic foot ulcers.<sup>251</sup> Phytosomes containing *Moringa oleifera* aqueous leaf extract were found to be non-toxic in NHDF cells till 3.0 mg/mL. The formulation at 1 mg/mL provided the shortest gap closure time (94.8% at 24 h) compared to the extract at the same concentration. Conversely, higher doses (1.25 and 1.50 mg/mL) did not reach statistically significant results, as well for lower doses.<sup>252</sup> A second in vitro study of NHDF cells evaluated a combination of gold nanoparticle (AuNP) and *Calendula officinalis* in phytosomal systems. The formulations reduced the interruptions of cell monolayer by about 42.2% for *Calendula* phytosomes and 58.7% for AuNP-*Calendula* phytosomes ( $p < 0.01$ ). The

combination did not show toxic effects up to 400  $\mu$ g/mL.<sup>33</sup> A complex of sinigrin-phytosome displayed beneficial effects on wound healing with respect to sinigrin alone, in HaCaT cells. After 42 h, the phytosome at 0.14 mg/mL completely solved the wound, whereas pure sinigrin reached only 71%, with negligible cytotoxicity towards cells.<sup>35</sup>

### Evidence of the Phytosomes Efficacy in Skin Cancer

Only two studies evaluated the potential effectiveness of phytosomes in fighting skin cancers. The first study showed a cytotoxic effect of the aforementioned sinigrin-phytosome complex in A-375 melanoma cells. At 0.14 mg/mL, the complex inhibited by almost 74% the cell viability, more than 46% displayed by free sinigrin, but only minimal toxicity was observed in non-tumoral HaCaT cells.<sup>35</sup> The second study considered the effect of silymarin in nanostructured lipid carriers (NLC) in vitro. Silymarin-NLC showed a higher inhibition (IC<sub>50</sub>: 21  $\mu$ g/mL) in cell viability of the human melanoma cell line (SK-MEL-2) in comparison to a non-specified phytosome commercial formulation (IC<sub>50</sub>: 26  $\mu$ g/mL).<sup>250</sup>

### The Phytosomes Effect in Musculoskeletal System

Pharmacological treatment of musculoskeletal dysfunctions is mostly based on non-steroidal anti-inflammatory drugs (NSAIDs) or analgesics; unfortunately, the therapy is often accompanied by several side effects. Among 16 studies regarding natural product-loaded phytosomes for treatment of musculoskeletal disorders, 62.5% were related to turmeric (*Curcuma longa*) extracts or curcumin, and 31.2% related to Indian Frankincense (*Boswellia serrata*) extracts.

One pilot study investigated the treatment of patients with osteopenia. Subjects with low bone density and no symptoms were treated for 24 weeks with the curcumin-based supplementation curcumin phytosome. The bone density of the heel, small finger, and upper jaw was assessed at 4, 12, and 24 weeks. A general improvement in bone density was observed in the group treated with 1 tablet/day containing 1000 mg of curcumin phytosome, whereas no significant changes were observed in the control group.<sup>253</sup> The same formulation, tested at the same dose either or not combined with other nutritional supplements and exercise, showed positive results in elderly subjects (>65 years) characterized by loss of strength, contributing to improving strength and physical performance.<sup>254</sup>

The efficacy of curcumin phytosome (1 g every 12 hours for 5 and 10 days) was tested on rugby players with different osteo-muscular pain conditions due to physical overload or traumatic injuries. The group taking curcumin phytosome (n=25) was compared with the group treated with conventional analgesic drugs (n=25). Pain and *functio laesa* were evaluated at different time points.<sup>255</sup> The analgesic effect of curcumin phytosome (2 g, corresponding to 400 mg curcumin) was demonstrated in patients with different chronic inflammatory diseases.<sup>256</sup>

Drobnic et al performed additional studies showing reduction of delayed onset muscle soreness following administration of phytosomal form of curcumin (1 g twice daily, for 4 days).<sup>257</sup> These studies suggest that curcumin-loaded phytosome could represent a useful remedy to counteract pain and osteo-muscular dysfunctions after intense physical activity. Other interesting studies in humans include the beneficial effects of mentioned phytosome (1 g/die) taken alone for 8 months<sup>258</sup> or curcumin phytosome (0.5 g/die) in combination with glucosamine (0.5 g/die) for 4 months<sup>259</sup> in patients with osteoarthritis. Administration of curcumin phytosome for 100 days (two 50-day cycle) at the dose 1000 mg/die for the first 30 days or 500 mg plus 300 mg  $\alpha$ -lipoic acid for the following 30 days of each cycle in patients with radiculopathy caused by spondyloarthritis or discopathy showed a reduction of pain rating.<sup>260</sup> The efficacy of a curcumin-phosphatidylcholine complex in children with uveitis associated with juvenile idiopathic arthritis, plus the conventional pharmacological treatment with immunosuppressive drugs, was assessed as well. The therapy in association with curcumin phytosome improved mild chronic anterior chamber flare and reduced inflammatory processes.<sup>261</sup>

The in vivo study by Farinacci et al demonstrated that curcumin-loaded phytosome possesses anti-inflammatory effects in mares and foals with chronic osteoarthritis or osteochondrosis through the downregulation of COX-2, TNF $\alpha$ , IL-1 $\beta$ , and interleukin 1 receptor antagonist (IL1RN) in leukocytes, although the effect was found statistically significant for the last two parameters.<sup>262</sup> Recently, the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) formulated an opinion on the beneficial effects of curcumin and the functioning of joints. Based on the data collected, the Panel concluded that the relationship between treatment with curcumin and efficacy in maintaining joint function cannot be claimed.<sup>263</sup>

Moreover, another phytosomal formulation containing only *Boswellia serrata* extract (BSE), named Boswellia phytosome, was clinically evaluated for the treatment of osteo-muscular pain in rugby players. The group took the supplement (500 mg/day of BSE) as co-adjuvant to standard therapy, whereas the control group followed only the standard therapy. Supplementation consisted of a single administration of 500 mg/day of Boswellia phytosome (2 tablets of 250 mg) for 5 days, followed by 23 days administration of 250 mg/day (1 tablet). Results by Visual Analog Scale for Pain (VAS Pain) and reduction of inflammatory biomarkers suggested that supplementation represents an effective therapy for the management of inflammation and pain, in addition to the conventional treatment.<sup>264</sup> Boswellia phytosome (250 mg/die) showed efficacy in reducing ankle sprains of mild severity (grade II) due to sports trauma in healthy subjects; parameters were evaluated after 3 and 7 days of treatment.<sup>265</sup> Boswellia phytosome efficacy in the treatment of musculoskeletal disorders has been recently reviewed.<sup>266</sup>

Another phytosomal formulation with Boswellia (200 mg), the rhizome of *Curcuma longa* (100 mg), the resin of *Commiphora wightii* (100 mg), and *Valeriana officinalis* extract (25 mg) were tested in 24 patients with osteoarthritis for 4 weeks. The main outcomes were the control of symptoms and functional capacity. The formulation showed promising effects in the management of osteoarthritis.<sup>267</sup>

A recent work investigated the effect of a phytosome (350 mg) loaded with *Zingiber officinale* (37.5 mg) and *Acmella oleracea* (7.5 mg) extracts against pain and inflammation in patients with moderate knee osteoarthritis. The study lasted 30 days. The formulation showed positive effects on physical activity and improvement of inflammatory biomarkers with no side effects.<sup>268</sup> Non-loaded liposomes have been studied for their ability to improve osteoblast adiposity and mineralization. Several liposomal formulations were synthesized using phosphatidylcholine, cholesterol and derivatives, and glycerol-phosphoethanolamine, and the effect on osteoblast differentiation and inflammatory condition was assessed. The study showed that neutral lipids induced less adiposity and maintain higher osteoblast mineralization than cationic lipids. In 7F2 mouse osteoblasts, phosphatidylcholine inhibited inflammation suppressing gene expression of COX-2 and MMP-3 induced by IL-1 $\beta$ , suggesting that this lipid is particularly relevant in the preparation of phytosomes against symptoms of bone diseases.<sup>269</sup>

## The Phytosomes Role in the Respiratory System Diseases

### The Phytosomes in Asthma and Bronchitis

A pilot study evaluated the effects of quercetin phytosome in addition to standard management (SM) in healthy subjects with mild-moderate asthmatic attacks and rhinitis. Subjects took 1 or 2 QFit tabs/day in association with SM or only this (control group). After 30 days quercetin phytosome + SM showed superior results compared with the control group, preventing and reducing daily and night symptoms, maintaining higher peak expiratory flow, and decreasing its variability, with a good safety profile.<sup>270</sup>

In a multicenter study, 32 asthmatic subjects were enrolled and received a combination of corticosteroids and beta-agonists, the standard management for patients with mild or severe persistent asthma. The subjects were randomized to receive *Boswellia serrata* phytosome 500 mg/day or no additional treatment for 4 weeks. Patients in phytosome group needed a lower number of inhalations compared to patients who receive only the standard therapy. The treatment with phytosome was well accepted, only mild-moderate adverse events such as insomnia and nausea were registered.<sup>271</sup>

Yu et al designed and developed a novel phytosome to promote pulmonary bioavailability of naringenin. One of the main lipids occurring in pulmonary surfactant, dipalmitoylphosphatidylcholine (DPPC), was efficiently used to deliver naringenin. The pharmacodynamic of naringenin-loaded DPPC phytosomes for dry powder inhalation (NPDPIs – 10 mg/rat, containing about 3 mg naringenin) was studied in rats with acute lung injury, and the relevant mechanisms of action were explored. These phytosomes have demonstrated protection against lung injury in rats when directly administered into the lungs. The data showed that NPDPIs alleviated pulmonary edemas with less fluid exudation and significantly down-regulated expression of cytokines, including COX-2 and ICAM-1. Moreover, naringenin and DPPC suppressed oxidative stress by upregulation of SOD activity in rats and the application of NPDPIs improved this effect.<sup>272</sup>

Singh et al have evaluated in vitro and in vivo a gingerol phytosome complexed with chitosan for treatment of respiratory disease. The sustained release of gingerol from the phytosome was demonstrated in vitro, as well as antioxidant and anti-inflammatory activities. Antimicrobial activity against the respiratory infective bacterial organisms was concentration dependent. The phytosome complex showed an important sustained-release profile and supported a better oral absorption of gingerol in a

pharmacokinetic study in vivo. The pharmacodynamic parameters showed an effective prolonged antibacterial and considerable anti-inflammatory activity against bacteria responsible for respiratory infections, in both Gram-positive and Gram-negative.<sup>273</sup>

### The Phytosomes Role in Lung Cancer

Curcumin formulated with phosphatidylcholine was evaluated as an antitumor agent in mammary gland tumor cell line (ENU1564) which were injected into the mammary fat of athymic nude mice. The effect of the phytosome was compared with free curcumin. Both substances did not affect tumor volume, but curcumin phytosome significantly decreased lung metastasis and the expression of MMP-9, a protein associated with progression and tumor invasion, including breast cancer.<sup>274</sup> Mao et al evaluated the biological activities of oral administration of grape seed phytosome in subjects participating in a lung cancer chemoprevention trial. Effects of phytosome on prostacyclin and 15-HETE eicosanoid pathways in human lung premalignant and malignant cells were determined. Results of this study support phytosome group as a chemo-preventive and anti-neoplastic agent against lung cancer.<sup>275</sup> In another study, oral administration of grape seed phytosome to athymic nude mice (200, 300, and 400 mg/kg/day for the group, containing GSE 56, 84, 112 mg/kg/day, respectively) down-regulated the oncomiRs miR-19a/b and miR-17-92 cluster host gene (MIR17HG) expressions. This was correlated with the in vitro grape seed phytosome activity in lung neoplastic cells, observed in the same study.<sup>276</sup> The maximum grape seed phytosome dose was well tolerated; at the end of treatment in bronchial biopsies was observed a significant decrease of Ki-67 labeling index (–55%), as well as a substantial reduction of bronchial histopathology grading, and significant downregulation of the expressions of miR-19a, miR-19b, and miR-106b in serum.<sup>277</sup>

### The Phytosomes Efficacy in Metabolic Syndrome

Metabolic syndrome (MS) is characterized by the presence of different metabolic dysfunctions including insulin resistance, type-2 diabetes, and obesity; the pathological condition is characterized by low-grade chronic inflammatory activity.<sup>278</sup> In a randomized, double-blind, crossover study, Vigna et al investigated the efficacy of grape seed phytosome on low-density lipoprotein (LDL) oxidation in heavy smokers. Enrolled subjects were treated for 4 weeks with 2 capsules twice a day, each containing 75 mg of a grape procyanidin extract, or the same amount of lactose used as placebo, and soy

phosphatidylcholine (phytosome). Subjects experienced negligible modifications of total cholesterol, triglycerides, and high-density lipoprotein-cholesterol (HDL-C) or LDL during treatment. However, thiobarbituric acid reactive substances (TBARS) amount was decreased, thus making this formulation particularly interesting in diseases characterized by stress oxidative.<sup>279</sup>

Mazzolani et al found that curcumin phytosomal formulation, administered as tablets (500 mg twice daily, representing a daily intake of 200 mg curcuminoids), showed improvement in visual acuity and optical coherence tomography retinal thickness in 11 patients with macular edema caused by diabetes. After 3 months of therapy, 84% showed improvement in visual activity and 16% stabilization, whereas no patients showed a reduction. Moreover, 92% of eyes considered in the open-label study showed a reduction of macular edema, and 8% stabilization, with no cases of increased pathology severity.<sup>280</sup>

The same curcumin formulation was administered with conventional therapy for 4 weeks at diabetic patients with microangiopathy and retinopathy. At the end of the treatment, patients showed a general improvement in microangiopathy. The amelioration of the veno-arteriolar response and the reduction of peripheral edema, which are indices of better microcirculation, were observed. At the retinal level, Steigerwalt and Snellen's scales showed improvement of retinal edema and visual acuity, respectively.<sup>281</sup> In another study by Di Pierro et al, 44 participants received 30 days of treatment with the curcumin-based product (800 mg/die curcumin and 8 mg/die piperine complexed with 20% sunflower phospholipid) or just phosphatidylserine (400 mg/die). Treatment with curcumin-induced weight loss and ameliorated percentage reduction of fat and BMI, suggesting that curcumin-based formulations may positively improve weight loss in overweight patients.<sup>282</sup> Recently, the same formulation (800 mg phytosomal curcumin containing 200 mg curcumin, 480 mg phosphatidylcholine, 120 mg phosphatidylserine, and 8 mg piperine), administered as two tablets/day, was assessed in 80 overweight subjects with fasting plasma glucose. The curcumin group showed a significant improvement in fasting plasma insulin and waist circumference.<sup>283</sup>

Several studies considered the effects of flavonoids-containing phytosomes on MS parameters. The phytosome formulation of green tea catechins showed improved blood lipid profile, blood pressure, and weight loss in subjects with borderline MS factors after a 24-week intervention study.<sup>284</sup> In another study, green tea phytosome (150 mg/dose) and 15 mg/dose piperine were administered to obese

women for three months to counteract weight regain after weight loss. Results of the study indicated that patients supplemented with the formulation maintained the body-weight reduction previously achieved than those receiving placebo.<sup>285</sup> These latest results confirm previous observations by Di Pierro et al in which the formulation, combined with low-calorie diet intake, increased weight loss, and reduced significantly BMI.<sup>286</sup>

Formulations including pure flavonoids quercetin or chrysin have been exploited at different levels on parameters related to MS. In particular, quercetin-loaded phytosome was orally administered at ovariectomized rats at 10 and 50 mg/kg for 4 weeks. Treatment decreased MDA and glucose levels, and improved lipid profile, with inhibition of pro-inflammatory mediators, such as TNF $\alpha$ .<sup>131</sup> Although these studies have not yet been confirmed in humans, another study showed that quercetin-loaded phytosome formulation administered with antidiabetics including metformin does not show significant drug interaction.<sup>287</sup> Moreover, better solubility and oral absorption by healthy volunteers were assessed with the quercetin phytosome formulation concerning the unformulated flavonoid.<sup>288</sup>

The dihydroxyflavone chrysin has been studied unformulated or complexed in different formulations including phytosomes. Complexation of chrysin with phospholipids was shown to improve solubility and promote glucose uptake in C2C12 cells, with nano-formulation CSP-1:3 (chrysin-loaded phytosome prepared with soy phosphatidylcholine at the molar ratio of 1:3) being able to stimulate peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) and glucose transporter type 4 (GLUT4) genes. The same study demonstrated that phytosome preparation may critically influence bioavailability and consequently biological activity.<sup>289</sup> Phytosome formulation containing silymarin improved bioavailability of flavonolignans ameliorating dyslipidemia associated with MS in hyper-triglyceridemic rats. After 4 weeks of treatment, 1% silymarin phytosome lowered plasma triglycerides and total cholesterol, while increased HDL cholesterol levels.<sup>290</sup> The same group demonstrated that the pure compound silybin seems to be the main component responsible for the observed effects, since it was able to increase HDL and decrease glucose and insulin levels.<sup>291</sup> In a randomized double-blind, placebo-controlled study, Mollace et al investigated the effects of a phytosome loaded with a standardized extract from bergamot (*Citrus x bergamia*), rich in naringin, in 60 patients with type 2 diabetes mellitus and hyperlipidemia. The authors described a significant reduction of fasting

plasma glucose, triglycerides, and LDL cholesterol associated with increased HDL cholesterol levels. Better absorption was observed for phytosome than the corresponding unformulated bergamot extract.<sup>292</sup>

Other studies using phytosomes loaded with phytochemicals or plant extracts include improvement of the hypoglycemic effect of the alkaloid berberine<sup>29</sup> and antidiabetic effects of a phytosome loaded with *Momordica dioica*, *Momordica balsamina*, and *Citrullus colocynthis*.<sup>293</sup> Moreover, phytosome containing extracts from mulberry (*Morus alba*) and ginger (*Zingiber officinale*), administered orally to male Wistar rats (50-100-200 mg/kg for 21 days), showed anti-inflammatory effects in adipose tissue; the formulation was able to decrease oxidative stress mediators and HDAC3, as well as increase PPAR $\gamma$  in the adipose tissue.<sup>294</sup> Non-loaded phytosomal formulations were also studied for their beneficial effects against obesity; soy phytosomal thermogel was found to possess anti-obesity activity in albino rats, whereas slight effects were observed on lipid profile.<sup>32</sup> Table 4 summarizes plant extracts and the relative range of doses used in clinical studies in different pathological conditions.

## Recent Patented Technologies on the Phytosomes

The leading dealer of nutraceutical ingredients developed a patented process for Phytosomes in 2011. Several scientists from academic and industries have found out innovative processes and developed phytosome formulations. The different summarized patents on phytosomes and their related innovative technologies are presented in Table 5.

## Marketed Phytosomal Products and Challenges to Commercialization

Phytosomes are considered as efficient nanocarrier delivery systems.<sup>310</sup> However, there is a long way from product development to a successful commercialization. Despite all advantages, a few final phytosomal products have been introduced into the markets.<sup>311</sup> After designing an effective formulation, proofing of safety is a primary barrier for the entrance of phytosomes into the market. Phytosomes have biologically neutral structures, so their introduction into the human body is acceptable without any concern about their safety or immunological

**Table 4** Plant Extracts and the Relative Range of Doses Used in Clinical Studies in Different Pathological Conditions

Plant Species	Dose (mg/Day)	Application	References
<i>Boswellia serrata</i>	200–4500	Central and peripheral nervous system	[194,199]
	250–500	Gastrointestinal system	[204–206]
	250–500	Musculoskeletal system	[264,265]
	500	Respiratory system	[271]
<i>Camellia sinensis</i>	150–300	Metabolic syndrome	[284,285]
	300	Genitourinary system	[222]
<i>Citrus x bergamia</i>	500–1000	Metabolic syndrome	[292]
<i>Curcuma longa</i>	2000	Gastrointestinal system	[201–203]
	900–2000	Genitourinary system	[236,237,242]
	2000	Integumentary system	[246]
	500–2000	Musculoskeletal system	[253–257,259–261]
	800–1000	Metabolic syndrome	[280–283]
	800–1000	Central and peripheral nervous system	[189,190]
<i>Ginkgo biloba</i>	360	Cardiovascular system	[175]
	120	Central and peripheral nervous system	[191,193]
<i>Silybum marianum</i>	2500–20,000	Genitourinary system	[234,235]
	188	Hepatoprotective effects	[214]
	2800	Genitourinary system	[223]
<i>Vaccinium macrocarpon</i>	24	Genitourinary system	[241]
<i>Vitis vinifera</i>	300–1200	Respiratory system	[275]
	150	Metabolic syndrome	[279]
	300	Immune system	[244]

**Table 5** Phytosomes Related Patents on the Developed Technologies

Sr. No	Title	Novelty/Innovation	Patent No. (Year of Grant)	References
1	Phospholipid complex of curcumin having improved bioavailability	Phospholipid complexes of curcumin provide a higher systemic level of parent agent than uncomplexed curcumin.	WO2009/101551 (2009)	[295]
2	Phospholipid complexes of olive fruits or leaves extract having improved bioavailability	Olive fruits/leaves extracts bioavailability enhanced using phospholipids complexes	EP1844785 (2007)	[296]
3	Compositions comprising <i>Ginkgo biloba</i> derivatives for the treatment of asthmatic and allergic conditions	Compositions of the fraction gained from <i>Ginkgo biloba</i> for the treatment of asthma and allergic conditions	EP1813280 (2007)	[297]
4	Treatment of skin, and wound repair, with thymosin $\beta$ -4	The formulation developed containing Thymosin $\beta$ 4 for wound healing	US/2007/0015698 (2007)	[298]
5	Oral compositions for the treatment of cellulite	Oral and cosmetic pharmaceutical Formulation containing <i>Centella asiatica</i> triterpenes, extracts of <i>Vitis vinifera</i> , and <i>Ginkgo biloba</i> flavonoids in the free or complexed form with phospholipids	US7691422 (2007)	[299]
6	Fatty acid monoesters of sorbityl furfural and compositions for cosmetic and dermatological use	The selected fatty acid monoesters of sorbityl furfural are lipophilic agents for specific anti hydroxyl radical activity	EP1690862 (2006)	[300]
7	Cosmetic and dermatological composition for the treatment of aging or photodamaged skin	The topical cosmetic or dermatological preparation containing at least one collagen synthesis-stimulating agent for anti-wrinkle treatment	EP1640041 (2006)	[301]
8	Soluble isoflavone composition	Isoflavone compositions enhanced the solubility, texture characteristics, taste, and color of the formulation	WO/2004/045541 (2005)	[302]
9	An anti-oxidant preparation based on plant extracts for the treatment of circulation and adiposity problems	The formulation developed having the plant extracts possessing the anti-oxidant activity for the treatment of phlebitis, hemorrhoid, arteriosclerosis, varicose vein, and elevated blood pressure	EP1214084 (2004)	[303]
10	Phospholipid complexes prepared from extracts of <i>Vitis vinifera</i> as anti-atherosclerotic agents	<i>Vitis vinifera</i> extract phospholipid complexes for the prevention and treatment of atherosclerosis.	US6297218 (2001)	[304]
11	Bilobalide phospholipid complexes, their applications, and formulations containing them	Complexes with synthetic or natural phospholipids and bilobalide (a sesquiterpene found in the <i>Ginkgo biloba</i> leaves) are revealed, as well as their formulation and application in inflammatory conditions and for the treatment of neuritic processes. This compound exhibited higher bioavailability than free bilobalide, hence it is applicable for parenteral and topical administration.	EP 0441279 (1991)	[305]

(Continued)

Table 5 (Continued).

Sr. No	Title	Novelty/Innovation	Patent No. (Year of Grant)	References
12	Complexes of neolignane derivatives with phospholipids, the use thereof, and pharmaceutical and cosmetic formulations containing them	Complexes of lipophilic extracts of <i>Krameria</i> or <i>Eupomatia</i> plant genus and some neolignanes isolated from the same extracts showed antibacterial, antimycotic, and antiradical activities. So it is a new active principle for the preparation of cosmetics and medicaments, as well as a good preservative in cosmetic preparations.	EP 0464297 (1990)	[306]
13	Complexes of saponins with phospholipids and pharmaceutical and cosmetic compositions containing them	The better bioavailability of saponins complexes with natural phospholipids suitable for cosmetic, pharmaceutical, and dermatological utilities	EP 0283713 (1988)	[307]
14	Complexes of flavanolignans with phospholipids, preparation thereof, and associated pharmaceutical compositions	This innovation consists of preparation lipophilic complexes of silidianin, silybin, and silicrist by non-conventional methods. Compared to individual flavanolignans, the resulted complex showed high gastrointestinal uptake followed by higher plasma levels. Because of this improved pharmacokinetic activity, the compound is applicable in the management of acute and chronic liver diseases.	EP 0209038 (1988)	[308]
15	Complex compounds of bioflavonoids with phospholipids, their preparation and use, and pharmaceutical and cosmetic compositions containing them	High lipophilia, improved bioavailability, and therapeutic properties obtained with complex compounds of flavonoids with phospholipids in comparison to free flavonoids.	EP 0275005 (1983)	[309]

reactions.<sup>312</sup> However, regarding their nano size, some parameters such as bioaccumulation, biocompatibility, metabolism, and excretion should be determined before their marketing.<sup>313</sup> Sou et al have successfully prepared a curcumin phytosome for intravenous application in rats, showed high accumulation in bone marrow and spleen tissues.<sup>314</sup> Another factor should be considered is the ability of phytosomes to merge with biological membranes and passively target normal cells. Hence, their actual biological effects should be determined in well-designed animal models as well as in clinical trial.<sup>312</sup> In this regard, different studies showed the biological safety of phytosomes.<sup>315</sup> Further to this, after designing a phytosome, pharmacokinetic and pharmacodynamic parameters should be assessed in animals and humans to prove their superiority rather than pure phyto-constituents. Finding the best dosage form to increase the absorption and efficacy of final product is another step in the way of marketing.<sup>316</sup> The other challenge is the production of phytosomes in a large scale. However, during scaling up, the characteristics of the product should be kept. This is related to the

practicality of laboratory protocol for in the industrial setup.<sup>313</sup> Although the manufacturing processes of many types of phytosomes are often simple, in regard to pH sensitive phytosomes, their low physicochemical stability makes their industrial production a challenge.<sup>312</sup> Phytosomes like other pharmaceutical products also should have reproducibility and should be checked for their quality during time.<sup>313</sup> Popularity is another factor in a successful commercialization of a product. Taken together, biocompatibility, low-priced and safety of natural products has grown preference of people for this type of therapies in the recent years. Moreover, commercialization of phytosomes is a rapid process due to simple manufacturing process and ease of promotion of phytosomal technology to industrial scale.<sup>317</sup> The enhanced bioavailability of polar phytoconstituents, advantages, and biological activities of phytosome formulations was explored by several pharmaceutical industries. The marketed phytosomes, their active constituents, the name of the industries, and specific indications are mentioned in Table 6.

Table 6 Therapeutic Applications of Phytosome Formulations

Sr. No	Trade/Common Name	Company Name	Phytoconstituents Complex	Biological Properties	References
1	Siliphos®	Indena	Silybin of <i>Silybum marianum</i>	Hepatoprotective and Antioxidant	[10,318]
2	Ginseng Phytosome	Natural Factors	Ginsenosides of <i>Panax ginseng</i>	Immunomodulator	[319]
3	Hawthorn Phytosome	Indena	Flavonoids of <i>Crataegus species</i>	Anti-hypertensive and Cardioprotective	[320]
4	Ginkgoselect® Phytosome	Herbal Factors	Flavonoids of <i>Ginkgo biloba</i>	Anti-aging & Brain vascular lining	[180]
5	Oleselect™ Phytosome	Indena	Polyphenols of <i>Olea europaea</i>	Anti-inflammatory and Antihyperlipidemic	[321]
6	Polinacea™ Phytosome	Indena	Echinacosides of <i>Echinacea angustifolia</i>	Immunomodulatory and as Nutraceuticals	[322]
7	Escin β sitosterol Phytosome	–	Escin β-sitosterol of horse Chestnut fruit	Antihyperalgesic	[248]
8	Ubiquosome® Phytosome	Indena	CoQ10	Essential endogenous cofactor for the electron transport chain in the mitochondria, antioxidant activity	[323]
9	Quercifit™ Phytosome	Indena	Quercetin	Sports nutrition, allergy seasons' discomforts control, antioxidant activity	[270]
10	Vazguard™ Phytosome	Indena	<i>Citrus x bergamia</i> Risso & Poit. - Fruit juice	Extremely effective in supporting healthy blood levels through the optimization of total cholesterol, c-LDL, c-HDL, triglycerides, and glucose levels	[324]
11	Casperome® Phytosome	Indena	<i>Boswellia serrata</i> Roxb. ex Colebr. – Resin	Healthy inflammatory response, Joint health, Gut health	[266,325]
12	Greenselect®/ Green Tea Phytosome	Indena	<i>Camellia sinensis</i> (L.) O. Kuntze – Leaf	Bodyweight balance, Antioxidant activity, Anti-Cancer, and Anti-oxidant	[286,326]
13	Leucoselect®/ Grape Seed Phytosome	Indena	<i>Vitis vinifera</i> L. – Seed	Cardiovascular and Anti-oxidant activities	[244,279]
14	Curcumin Phytosome	Indena	<i>Curcuma longa</i> L. - Rhizome	Joint health, Healthy inflammatory response, Soothing	[257]
15	Virtiva®/ Ginkgo biloba Phytosome	Indena	Ginkgo flavonglycosides like ginkgolides, bilobalide	Improve cerebral insufficiency	[327]
16	18 β-glycyrrhetic acid Phytosome	Indena	18 β-glycyrrhetic acid from the rhizome of Licorice	Soothing, Anti-inflammatory activity	[328]

17	Visadex Phytosome	Indena	Visnadin from <i>Amni visnaga</i>	Improve microcirculation	[329]
18	Polinacea Phytosome	Indena	<i>Echinacea angustifolia</i> Root	Improve immune system	[330]
19	Lymphaselect	Indena	<i>Melilotus officinalis</i>	Used for chronic venous insufficiency of the lower limbs	[174]
20	Naringenin Phytosome	-	<i>Citrus aurantium</i>	Anti-oxidant, Anti-inflammatory effect	[272]
21	Vazguard™/Naringin Phytosome	Indena	Bergamot extract	Anti-oxidant, treatments of cardiometabolic disorders	[292]
22	Xanthones Phytosome	-	<i>Swertia alternifolia</i>	Anti-oxidant	[331]
23	Mirtoselect®/Anthocyanose Phytosome	Indena	<i>Vaccinium myrtillus</i>	Antioxidant, anti-inflammatory, diabetic retinopathy	[332]
24	Centevita®	Indena	Asiatic acid, madecassic acid from <i>Centella asiatica</i>	For skin disorders, antiulcer, wound healing, hair falling	[182]
25	Rhizoma paridis Phytosome	-	Rhizoma paridis from Paris polyphylla, steroidal saponins	Antitumor activity, immunity adjustment, antiviral and anti-inflammation	[333]
26	Berberine-phospholipid complex-based phytosomes	-	Berberine	Antidiabetic	[29]
27	Evodiamine phospholipids complex	-	Evodiamine	Anti-tumor	[28]
28	Ginseng Phytosome	Indena	<i>Panax ginseng</i>	Nutraceutical, immunomodulatory	[334]
29	Soyselect®/ Soybean extract Phytosome	Indena	Glycine max extract	Anti-angiogenic, anti-cancer, cardioprotective, immunostimulatory and anti-hyperlipidemic	[32]
30	Cucurbita Phytosome/ Tocopherol, carotenoids Phytosome	-	<i>Cucurbita pepo</i>	Anti-inflammatory, Prostatic hyperplasia	[335]

## Conclusion

With the rise in the number of recently discovered phytochemicals, research will be brought up to date on their medical benefits in a biological environment. However, low solubility and sensitivity to degradation restrict the application of these compounds in food and pharmaceutical products. At this stage, gaining insight into vesicular drug delivery systems could help to improve these characteristics. Vesicles are shown to be very promising delivery systems for various beneficial phytochemicals at a cellular level, because of their remarkable entrapment capacity, biocompatibility, and safety. Among vesicular drug carriers, phytosomes form a complex between phytochemicals and phospholipids, which results in the improvement of absorption and bioavailability of bioactive molecules, together with improved overall compound stability. Liposomes, transfersomes, niosomes, and ethosomes are the most used nanocarriers for phytochemicals, which are characterized by different dimensions, release efficiency, or preferential target (eg, transfersomes and ethosomes for topical application). Similarly, nano-phytosomes are one of the newest lipid-based vesicles with lower dimensions, a development to further boost the transport of plant-based nutraceuticals. Each formulation must be adequately characterized to ensure a high safety profile and meet reproducibility standards, through analysis of physical measures that give information on both dynamics of release and formulation stability.

This review provides an overview of biological activities of phytosomes both for commercial and non-commercial products. The set of collected studies shows a general advantage in the use of these formulations to improve the bioavailability of bioactive phytochemicals, allowing a reduction in dosage, compared to non-formulated compound, or greater biological activity. All the considered human systems are characterized by the presence of at least a clinical study. However, the superiority of the formulation has only rarely been investigated in comparison with its components in clinical trials. Exceptions are studies on the bioavailability of quercetin<sup>288</sup> and bergamot<sup>292</sup> and a comparison between the anti-adhesive activity of urine of subjects following oral consumption of cranberry extract;<sup>241</sup> in all the cases, the formulations gave higher values. Among the sources of phytochemicals *Curcuma longa* and *Silybum marianum* have collected most of the clinical evidence, with positive effects, except for silibinin in the management of prostate cancer, which yielded only marginal results. Overall, clinical studies are currently

insufficient to draw conclusions on biological activities of individual preparations, but the overall evidence for these formulations is encouraging and invites the researchers to continue investigations in this field. In the future, clinical studies on standardized products that show superior efficacy compared to non-formulated components or extracts will be fundamental to drive attention to these technologies.

## Disclosure

The authors report no conflicts of interest for this work.

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