Phytosomes: A promising delivery system for anticancer agents by using phytochemicals in cancer therapy

Pooja Dave1, Rupal Jani1**, G S Chakraborthy1, Kaushal J Jani2, Vijay Upadhye3, Danial Kahrizi4, Mushtaq Ahmad Mir5, Samra Siddiqui6, Mohd Saeed7, Tarun Kumar Upadhyay**

1Parul Institute of Pharmacy & Research, Parul University, Vadodara, 391760 Gujarat, India
2Formulation Development, Non-Oral Department, Sun Pharmaceutical Industries Limited, Tandlja, Vadodara, Gujarat, India
3Department of Life Sciences, Parul Institute of Applied Sciences and Research and Development Cell, Parul University, Vadodara 391760, Gujarat, India
4Agricultural Biotechnology Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
5Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid university, Abha, 61421, Saudi Arabia
6Department Health Services Management, College of Public Health and Health Informatics, University of Hail, Hail P.O. Box 2240, Saudi Arabia
7Department of Biology College of Sciences, University of Hail, Hail, Saudi Arabia

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ABSTRACT

Plant extracts have been shown to be effective in treating a variety of ailments. However, their hydrophilic nature and unique chemical structure have caused significant hurdles due to their low bioavailability. Phytosomes technology is used to improve the absorption of phytoconstituents that are difficult to absorb. Among the leading death in society is malignancy. The aforementioned consumes remained a big issue for modern chemotherapy since it has yet to be treated efficiently. The goal of this study is to outline the most recent research on the potential use of phytosome complexes for cancer therapy, as well as the formulation processes and mechanism of transportation through pyrosomes. Nanotechnology has paved the way for cancer therapy by altering key features of medications and their carriers. Novel drug delivery systems are used to transfer antitumor drugs to a particular site via different nanostructures. Among several unique drug delivery systems, phytosomes are a creative way to transfer phytoactive compounds to the site of action, and several phytosomes formulations are now being used in clinical settings. Phytoconstituents' anti-cancer activities are increased by phytosomal formulations.

Keywords: Cancer, Anticancer agents, Phytosomes, Nanoparticles, Liposomes, Drug delivery system

Introduction

A phytosome is a phospholipid and natural active component combination. When administered topically or orally, phytosome improves the absorption of plant extracts (1). Herbal extract is bound by phospholipids in phospholipid complexes called phytosomes, sometimes known as hermosomes (2). It refers to a lipid-encased vesicle-based drug delivery technique that uses phytochemical constituents. By enhancing phytoconstituents absorption through the GIT, phytosomes increase phytoconstituents bioavailability (3, 4). In contrast to liposomes, which include numerous phosphatidyl choline units around water-soluble components, phytosomes contain phytoconstituents and phospholipids in a 1:1 or 1:2 ratio (4). Lipophilic vesicular drug delivery systems known as phytosomes are highly soluble in non-polar solvents, have a predetermined melting point, and are only weakly soluble in lipids. Different polymeric and lipid-based nanocarriers are represented in Figure 1.

Therapeutic impact of phytotherapeutics

Phytotherapeutics have therapeutic relevance. Many people prefer herbal remedies over traditional pharmaceuticals for symptom relief. The pharma industry has consequently lately concentrated on finding emerging treatment chemicals originating from herbal remedies. Plant-derived novel molecular entities account for around 25% of FDA-approved natural new molecular entities. A benzyl isoquinoline alkaloid known as morphine, which was discovered in the Papaver somniferum and authorized for usage in 1827 (1) is the first plant-derived NME. Its...
effects on the neurological system are mostly attributable to presynaptic neurons’ suppression of neurotransmitter emission. After the discovery of morphine, more active substances were found, including an alkaloid derived from the pacific yew plant called paclitaxel (Taxus brevifolia) (3). Paclitaxel prevents the dynamic microtubule disintegration processes necessary for optimal mitotic spindle formation as well as chromosomal segregation during cell division by binding to -tubulin and stabilizing the microtubule polymer (4). Among other malignancies, paclitaxel is used to treat ovarian, breast, lung, pancreatic, and Kaposi sarcoma caused by human herpes virus-In addition, Catharanthus roseus yielded the indole alkaloids vinblastine and vincristine (5-8). Several broad-spectrum chemotherapeutic drugs inhibit microtubule polymerization including M-phase cell cycle inhibition by binding to tubulin at a different site beyond paclitaxel. Inappropriately, various therapeutic interventions, such as physicochemical limitations including low solubility and instability, make less effective delivery of phytotherapeutics. Pharmacokinetic issues, for instance, bioavailability and absorption issues can also cause issues in clinical studies. Furthermore, the challenges of phototherapeutic translation in the pharmaceutical business, such as batch-to-batch fluctuation and poor efficacy, continue to be significant (8).

The importance of herbal remedy in the treatment of various maladies, in addition to the obstacles it faces, may be seen from the few instances given above. The concern has portrayed attention to the need for it to be provided in a safe and significant manner. Innovative delivery technologies are widely useful by using phytochemicals, for example, vesicular drug delivery systems, and particulate drug delivery systems. These administration methods increased pharmacological efficacy, delivery speed, physical and chemical stability, and bioavailability, all of which contributed to a longer half-life. Mechanisms of action of phytosomes that can be utilized in cancer therapy are described in Figure 2 and Figure 3 showing the detailed accounts of phytosomes drug loading strategies and mode of action in anticancer drugs.

**Phytosomes preparation**

Phosphatidylcholine and phytoconstituents are mixed in a 1:1 ratio in an aprotic solvent to make phytosomes. The ratio of phospholipid phytoconstituents in phyto-phospholipid complexes is in the range of 0.5-2 mole. The best phospholipid-to-phytoconstituents ratio is 1:1. Soya-lecithin phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine are the most often used phospholipids. Chemical linkages bind the molecules of phospholipid to phytoconstituents, according to spectroscopic analysis (3, 4). In Table 1 we have shown different evaluation parameters used for phytosomes.

**Physicochemical Characteristics**

**Solubility**

It is possible to evaluate the solubility of an excess of the drug in different types of solvents according to drug’s nature and BCS Classification. For example, distilled water, acetone, phosphate buffer, acetate buffer, ethanol, methanol and many more.

**Particle size and size distribution**

Particle size distribution of produced phytosomes may be studied by dispersing them in an alcoholic solution (iso-

<table>
<thead>
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<th>Parameters</th>
<th>Techniques</th>
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<tbody>
<tr>
<td>Phytosomes size, shape, and particle size distribution</td>
<td>Optical Microscopy, Flow cytometry, Sedimentation by centrifugation, SEM, Tem, DLS, AFM, DSC</td>
</tr>
<tr>
<td>Compatibility and chemical composition studies</td>
<td>FTIR, NMR, HPTLC, TGA, LCMS, GCMS, TLC</td>
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<tr>
<td>Surface Charge</td>
<td>Zeta potentiometer, Lessor Doppler, Velocimetry, Electrophoresis</td>
</tr>
<tr>
<td>Stability analysis lamellarity</td>
<td>NMR, Small angle x-ray scattering, SANS, Electron microscopy method, TGA, DSC, DLS, HPLC, UV-vis.</td>
</tr>
<tr>
<td>Drug release behavior and Encapsulation efficiency</td>
<td>UV-visible, HPLC, TLC, HPTLC, dialysis, enzymatic assay in situ methods, gel electrophoresis</td>
</tr>
<tr>
<td>Optimization</td>
<td>By applying various Design of Experiments (DoE) for instance, 2¹ factorial design, Box Behnken design</td>
</tr>
</tbody>
</table>

![Figure 2. Phytosomes for cancer therapy.](image)

![Figure 3. Phytosomes drug loading strategies and mode of action in anticancer drugs.](image)
propyl alcohol) and analyzing them using a size analyzer (zeta sizer).

Stability of pharmacosomes

It is essential to assess the stability of the complex by contrasting the spectra of the complex in the solid state with the frequency of diffusion of microscopic particles suspended in water.

Dissolution studies

In vitro dissolution tests are carried out on a variety of mediums using various pH values utilizing conventional dissolution apparatus. The effectiveness of the active substances is considered while evaluating the outcomes.

Morphological Studies

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) can be used to investigate the morphological characterization of pharmacosomes. The purity grade of phospholipid as well as process factors such as rotation speed, the vacuum used, and the technique employed might impact the form and size of pharmacosomes. Pharmacosomes made with low-purity lipids provide a greasy result. Pharmacosomes made with high-purity lipids are susceptible to oxidative breakdown.

Surface tension activity measurement

Different approaches, such as the Du Nouy ring tensiometer technique, will use to determine the surface tension activity of aqueous solutions of phytoconstituents.

Crystallinity

Due to complexation, the crystallinity of phytoactive chemicals is lost, resulting in a balance of hydrophilicity and hydrophobicity. The formulation’s crystallinity will be determined by using differential scanning calorimetry (DSC) and x-ray diffraction (XRD). The crystalline nutraceutical moiety is shown by a prominent peak in DSC thermograms also at increased melting temperatures. The appearance of a wide peak is due to the structure of phytoActive chemicals, which has a melting point that is significantly lower as compared with original natural products. The widespread peak shows a decrease in the crystallinity of phytoactive compounds. Diffraction of angles (2) of respective components, phospholipids, phytoconstituents encapsulant, in addition, phagosomal complexes are compared using the x-ray diffraction technique. Loss of crystalline peaks of encapsulant may be determined by XRD, indicating that their interaction with phospholipids is acceptable. Power XRD (PXRD) is a cardinal approach to studying changes in the inner state of drug/nutrient crystals. PXRD examination of a phytosome containing quercetin displays intense diffraction peaks, indicating quercetin is crystalline. The existence of a single wide peak explains the amorphous character of phospholipids. The elimination of quercetin's crystalline peaks, for example, may characterize the arrangements of quercetin and phospholipid once they've been added to the phytosome complex or physical combination.

This represents quercetin is disseminated (molecularly or amorphously) in a phospholipid matrix (9). Other studies on the phytosome complexes of baicalein, aspirin and rifampicin (9-11) corroborate similar findings. When quercetin-phospholipid complexes develop, the crystal-line peaks of quercetin vanish from XRD graphs. As a result, PXRD data confirms the DSC study, demonstrating decreased medication or nutraceutical crystallinity after application.

Nuclear Magnetic Resonance (NMR)

The phytosome complex, PC, and pure forms of phytoactive chemicals may be distinguished enough by comparing 1H-NMR and 13C-NMR spectra. The peaks of fatty acid chains are essentially unaltered, suggesting that they are enfolded around the phytoactive chemical, resulting in the formation of a lipid compatible covers phytosomal polar component’s shield which allows the complex to be dissolved in solvents containing different conductivity. Bombardelli et al. investigated the 1H-NMR spectra of catechin but also its stoichiometric phytosomal complex with PC. In nonpolar liquids, it was a notable alteration in the 1H-NMR peak, with no summation of the individual molecule peaks. The peak of the tail of phospholipids remains unaltered in 1H-NMR spectra of various phytosome complexes, showing that this section does not engage in any chemical interactions and the central choline part that makes a connection to the phytoconstituents is enveloped. The 13C-NMR is widely used to confirm the sorts of interactions that occur throughout the complex building process. The 13C-NMR spectra of catechin and associated phytosome complex revealed that all flavonoid carbons vanished. The extended maxima are caused by the choline component, whereas the majority of the fatty acid chain resonances remain at their peaks. Silibinin interferes with mitochondrial metabolic pathways, according to 1H, 13C, and 31P-NMR investigations, and this is enhanced by using phytosome technology (12, 13).

Entrapment Efficiency (EE)

The ultracentrifugation technique can be used to evaluate the EE of phytoconstituents loaded with phytosomes. The EE percent can be determined via ultracentrifugation at lower rpm-longer intervals or higher rpm-shorter times. Furthermore, the supernatant should be calculated in order to detect phytoactive chemicals using UV-Visible spectroscopy or, more accurately, HPLC (14).

Retention Time

In terms of chromatography, HP-TLC has been described as a straightforward approach to determining the identity of phytosomes. When phytosomes are studied singly, they have a different retention period than phospholipids and phytoconstituents, indicating that a new complex has formed (15).

Applications of phytosome in cancer therapy

Plant-derived compounds have been proven to have a variety of anti-cancer properties (16-18). Herbal extracts' less solubility of lipids and high molecular size, which restrict their ability to pass through phospholipid-based cell membranes, are mostly too responsible for their low bioavailability (19). New preclinical as well as clinical studies have confirmed that an anticancer plant-derived chemical, packaged in an appropriate herbal delivery system like nano phytosomes, may overcome the constraints of herbal extracts’ low absorption, which confines its clinical uses in cancer treatment.
**Silibinin Phytosome**

After encapsulating them in nano phytosomes to improve their low bioavailability, Ochi et al. assessed the effects of two plant-based anti-cancer drugs, glycyrrhizic acid and silibinin, on hepatocellular carcinoma (HCC) cell lines (HepG2). By lowering N-nitrosodimethylamine, silibinin, a naturally occurring substance generated from silymarin, has been demonstrated to have anti-cancer activities in hepatocellular carcinomas (20). Glycyrrhizic acid, a natural treatment derived from Glycyrrhiza glabra (L.), has been shown to have anti-cancer benefits due to its efficiency as an MMP inhibitor as well as DNA protection in malignant cells (3). 14 percent (w/w) of silibinin and 88 percent (w/w) of glycyrrhizic acid were released over the course of 48 hours in an in vitro release research. Co-encapsulated nano-phytosomes of silibinin and glycyrrhizic acid were three times more efficient than individual silibinin (25 percent w/v) and glycyrrhizic acid (75 percent w/v) in a cell viability experiment on HepG2 cell lines. Glycyrrhizic acid’s synergistic actions allowed phytosome technology to be used to create co-encapsulated systems that boosted the therapeutic benefits of silibinin. Alternatively put, phytosomes enhance the bioavailability of silibinin (21)[59]. Reducing the expression of the HIF-1 protein (22), revealed possible inhibitory actions on individual PC-3 and LNCaP cells (human prostate cancer cell lines) as an anticancer drug (23). Recent studies have demonstrated that combining silibinin takes additional anti-cancer drugs (such as doxorubicin). Recently, some studies have confirmed that combining silibinin with other anti-cancer drugs (such as doxorubicin (24), mitoxantrone (25), cisplatin, carboplatin (26), etc.) increased its effectiveness against prostate cancer. Flaig et al.(26) examined the effects of a higher dose of silibinin administered orally and without phytosomes on prostate tumor patients two weeks previous to prostatectomy. Even though silibinin phytosomes can produce significant plasma concentrations, the amount of silibinin in prostate tissue is rather low. The brief research period may be to blame for silibinin’s underwhelming effects on prostate cancer, but phytosome size as long as it's nanoscale could possibly play a part (27). Matteo Lazzeroni et al.(27) evaluated the pre-surgical special effects of oral Silybin nanophytosomes (SNPs) administration to patients with initial breast cancer in a distinct study. Despite the fact that all of the patients completed their treatment plan, no side effects were observed. In accordance with the findings of this study, the delivery of SNPs in cancer tissues was almost four times greater than that of free silibin.

**Sinigrin Phytosome**

Human health depends on the proper management of malignant wounds, especially those caused by skin cancer. Agents that treat wounds and kill cancer cells simultaneously are in great demand. One of the main naturally occurring glucosinolates from the Brassicaceae family, sinigrin, has been found to have anticancer effects (28, 29). Mazumder et al.(2) investigated the antitumor effects of Sinigrin's phytosomal formulation on A-375 melanoma cells and the wound healing advantages of Sinigrin on normal human keratinocytes cells (HaCaT). Low cytotoxic effects were found under in vitro cytotoxic experiments on healthy cells (HaCaT), and sinigrin phytosome complex significantly outperformed free sinigrin on A-375 cell lines. HaCaT cells were used in wound healing trials that showed a 50% increase in wound closure at various times and concentrations. Sinigrin phytosomes were found to increase the therapeutic effect in the treatment of cancer also malignant wounds (2).

**Mitomycin Phytosome**

The natural substance mitomycin C (MMC), which has an aziridine ring and a carbamoyl chain, has potent anticancer capabilities and is used to treat a variety of cancers (30). A high rate of absorption into the systemic circulation, which leads to a drop in the plasma concentration of medication in effect-relevant locations and, as a result, a reduction in MMC's therapeutic effects, is the biggest barrier to its clinical use. Hou et al. had done their work in Mitomycin C soybean loaded phytosomes PC (MMC SPC) complex delivery system with a mean particle size of 210nm in order to address this problem. In an in vitro release study, it was discovered that MMC was released continuously after its first burst. These results suggested that phytosomes might successfully protect MMC from rapid absorption into systemic circulation by increasing its lipophilicity. In an in vivo anticancer investigation, the tumor suppression rate of phytosomes loaded with MMC was six times greater than that of MMC injection in H22 solid tumor-bearing model mice (31).

**T. arjuna Phytosome**

T. arjuna (TA), a member of the Combretaceae family, has antimutagenic and anti-cancer effects, and its bark is rich in flavonoids (32). The main problem with TA bark extract is that it has a limited bioavailability, which prevents it from being used therapeutically. Shalini et al. developed a nano phytosome complex of methanolic extract of TA bark (with diameters ranging from 30 to 80 nanometers) to make up for TA’s limited bioavailability and investigated its antiproliferative effect on human breast cancer cell lines (MCF-7). In a cell proliferation test on MCF-7 cells, TA bark phytosome performed better than TA bark extract (nearly 1.6 times more effective than free TA bark extract) (33).

**Luteolin Phytosome**

Luteolin (Lut), a natural component, has been found to have anti-cancer activities by acting on multiple molecular targets to kill cancer cells because of its potency to inhibit the Nrf2 signalling pathway selectively and sensitize non-small lung cancer cell lines (NSCLC A549) to anti-cancer agents (34, 35). High levels of Nrf2 expression have been seen in cancer cells, and research has implicated this protein with drug resistance (36). By preventing Nrf2-mediated signaling, Sabzichi et al. were able to make human breast cancer cells (MDA-MB 231) more susceptible to the chemotherapy drug dox. Cytotoxicity tests on MDA-MB 231 cells showed that nano-phytosomes of luteolin in combination with doxorubicin performed better than nano-dox alone. In conclusion, phytosomes may improve the therapeutic effects of dox by boosting luteolin's effectiveness at inhibiting Nrf2 and luteolin's bioavailability (37).

**Curcumin Phytosome**

Turmeric, sometimes referred to as Curcuma longa, is a traditional plant that has been used for thousands of years. Curcumin, demethoxycurcumin, and bisdemethoxycurcu-
Phytosome in cancer therapy: challenges and future aspects

Despite the apparent potential of phytosome technology, only a few anti-cancer studies have been conducted using phytosome as a carrier in cancer therapy. As a result, only a few products, such as Meriva® (curcumin phytosome) and Siliphos® (Silybin phytosomes) (53), have entered the market. Another important element in this respect is the larger-scale manufacture of nano phytosomes. Phytosomes' simple and quick production techniques make them more potent to scale up, even if they have yet to be used on an industrial scale. The primary reason for the non-industrial scale-up may be the pH sensitivity of phytosome structures. To commercialize this nanocarrier in the future, this important barrier that influences the physicochemical stability of phytosomes must be removed.

An exciting aspect of phytosomes is the potential for and availability of manufacturing methods that are suitable for food, which minimize any potential adverse effects of phytosomes produced by non-food-grade technologies. Food-grade phytosomes may be made using a combination of food-grade solvents, such as ethanol, and PCs are approved as food-grade additives (14, 54). In conclusion, despite the limitations of the manufacturing process, phytosomes are still a promising option for industrial cancer production for cancer-related purposes.

Nanotechnology has had a significant impact on anti-cancer drug delivery because of its advantages over conventional delivery systems, including drug distribution, controlled drug release, avoiding reticuloendothelial system elimination, and passive targeting to cancerous tissues via the enhanced permeability and retention (EPR) effect. As a result, the FDA has authorized a few nanoparticulate drug delivery devices for cancer treatment. Among the different nanocarriers that have been introduced, lipid-based nanoparticles have a number of benefits over other drug carriers, including biocompatibility and biodegradability, low cost and raw material obtain ability and long antiquity of research. Herbal medications, for instance, flavonoids, have in recent times sparked a lot of interest in cancer treatment, but their poor oral bioavailability prevents them from being used in clinical trials. Furthermore, hydrophilic flavonoids and/or hydrophilic anti-cancer drugs may not have enough capability in lipid-based nanoparticles. Phytosomes circumvent these challenges by providing polar active components with a large number of complexation sites. Phytosomes are a good vehicle for the administration of medicine because their phospholipid and cholesterol cores resemble biomembranes in a big way. These lipids are tolerated when introduced into human tissues without triggering antigenic or pyrogenic responses, possibly eliminating the issues caused by polymer incompatibility. Additionally, phytosomes have biologically inert properties and seldom elicit immune reactions. To fully comprehend these potentials, more research is required to evaluate the efficiency and toxicity of nano phytosomes in large animal models and subsequently in cancer patients in Phase I/II clinical trials. The ability of phytosomes to bind to bilayer phospholipid biological membranes appears to be a significant obstacle that has to be addressed cautiously, even if nanoparticles have the potential for passive targe-

**Table 2.** Nanovesicles used in anticancer activity by entrapped herbal formulation.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Vesicular Structures</th>
<th>Results</th>
<th>References</th>
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<tbody>
<tr>
<td>Quercetin</td>
<td>Phytosomes</td>
<td>Improved anticancer activity on MCF-7 cell line</td>
<td>(44)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Liposomes</td>
<td>Permeation rate is higher and easily crosses the blood-brain barrier</td>
<td>(45)</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>Ethosomes</td>
<td>Drug loading efficiency is 99% and has high cytotoxic activity against the MCF-7 cell line.</td>
<td>(46)</td>
</tr>
<tr>
<td>Curcumcarvi</td>
<td>Niosomes</td>
<td>Better effect of anticancer activity against MCF-7</td>
<td>(36)</td>
</tr>
<tr>
<td>Leuteolin</td>
<td>Phytosomes</td>
<td>Improved anticancer activity on MCF-7 cell line</td>
<td>(47)</td>
</tr>
<tr>
<td>Lawsome</td>
<td>Niosomes</td>
<td>Enhances entrapment efficiency, sustained release and antitumor activity</td>
<td>(48)</td>
</tr>
<tr>
<td>Gombogic acid</td>
<td>Liposomes</td>
<td>Used in breast cancer to inhibit cell growth</td>
<td>(49)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Liposomes</td>
<td>Used in colonic antioxidant</td>
<td>(50)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Liposomes</td>
<td>To treat lung cancer and inhibit cell growth against A549</td>
<td>(51)</td>
</tr>
<tr>
<td>Camptothesin</td>
<td>Liposomes</td>
<td>Phosphatidylcholine with a disulfide bond has a great effect on tumor therapy</td>
<td>(52)</td>
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</tbody>
</table>
ting. This ability of phytosomes leads to the passive targeting of cancerous sites while non-specific drug distribution to healthy tissues. This is a critical barrier because adverse drug and excipient reactions might harm healthy cells and organs. In-depth investigations of innovative active targeting approaches (such as antibody- or peptide-targeted delivery) must be created and applied to overcome these concerns in order to increase anticancer efficacy while minimizing damage to healthy tissues. Aside from recent developments in industrial-scale vesicular system fabrication, like extrusion technologies, which offer promising prospects for the commercial fabrication of these systems, a threat to this development may come from the high cost of raw materials like PEGylated soy phosphatidylcholine. The concept and technique behind the production of nano phytosomes have a promising future in their applications, despite the enormous difficulties involved. It is anticipated that nano phytosomal delivery methods for cancer therapy will progress and grow in the near future. In Table 3 we have enlisted different marketed Preparations of Phytosomes that can be utilized in cancer therapy.

Conclusions

The health-promoting chemicals in herbal products, such as flavonoids and other phenolic compounds, are credited with their efficacy. As a result, it appears that having suitable delivery mechanisms that can transport sufficient amounts of active components to the body is critical. Phytosomes are new medication delivery technologies that improve the bioavailability of phytoconstituents and other naturally occurring plant-based chemicals in the gastrointestinal tract. Phytosomes have distinct benefits over other traditional nanodelivery carriers that cannot be denied. The phytosome formulation technique is simple to implement and may easily be scaled up to commercial levels. Phytosome technology has the potential to be used in the nano-formulation of nutraceuticals in the future since it is a promising candidate for bringing hydrophilic plant chemicals to cancer therapy.

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Interest conflict
No conflict of interest.

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