the target tissue; therefore, phytosomes are suitable as targeted delivery system.

- High entrapment efficiency as the drug itself is in conjugation with lipids in the vesicles.
- No problem in drug entrapment while formulating phytosomes.
- Phosphatidylcholine nourishes the skin besides acting as a carrier as it is an integral part of plasma membrane.
- Phytosomes are also better than liposomes in terms of topical delivery.
- Phytosomes have significantly greater therapeutic benefits.
- Phosphatidylcholine also acts as a hepatoprotective and imparts a synergistic effect when hepatoprotective substances are incorporated.
- Low solubility in aqueous media allows the formation of stable semisolid dosage form.
- Promotes liver targeting by increasing the solubility in bile salt.

PROPERTIES OF PHYTOSOMES

Physico-chemical properties

Phytosome is a complex between a natural product and phospholipids obtained by a reaction of stoichiometric amounts of phospholipids and the substrate in an appropriate solvent. On the basis of spectroscopic data, it has been observed that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e., phosphate and ammonium groups) and the polar functionalities of the substrate. On treatment with water, phytosomes acquires a micellar shape forming liposomal structure which can be concluded by comparison of the NMR of the complex with that of the pure precursors. The signals of the fatty chain are almost unchanged. Such evidences referred that the two long aliphatic chains are wrapped around the active content, producing a lipophilic envelope, which shields the polar head of the phospholipid and active constituents [7].

Biological properties

Phytosomes are improved forms of herbal formulations that are better absorbed, utilized and produce better results than conventional herbal extracts. The increased bioavailability of the phytosome over the non-complexed herbal derivatives has been demonstrated by pharmacokinetic studies or by pharmacodynamic tests in experimental animals and in human subjects [8].

METHODS OF PREPARATION FOR PHYTOSOMES

Anti-solvent precipitation technique

The specific amount of plant extract and phospholipid were taken into a 100 ml round bottom flask and refluxed with 20 ml of dichloromethane at a temperature not exceeding 60°C for 2 h. The mixture was then concentrated to 5-10 ml. Hexane (20 ml) was added carefully with continuous stirring to obtain the precipitate which was filtered and collected and stored in desiccators overnight. The dried precipitate was crushed in mortar and sieved through #100 mesh. Powdered complex was placed in amber colored glass bottle and stored at room temperature [9].

Rotary evaporation technique

The specific amount of plant material and phospholipid were dissolved in 30 ml of tetrahydrofuran in a rotary round bottom flask followed by stirring for 3 hours at a temperature not exceeding 40°C. Thin film of the sample was obtained to which n-hexane was added and continuously stirred using a magnetic stirrer. The precipitate obtained was collected, placed in amber colored glass bottle and stored at room temperature.

Solvent evaporation technique

The specific amount of plant material and phospholipids were taken into a 100ml round bottom flask and refluxed with 20ml of acetone at a temperature 50-60°C for 2h. The mixture was concentrated to 5-10 ml to obtain the precipitate which was filtered and collected. The dried precipitate phytosome complex was placed in amber colored glass bottle and stored at room temperature.

Ether-injection technique

In this technique, the drug lipid complex is dissolved in an organic solvent. The mixture is then slowly injected into a heated aqueous agent, resulting in the formation of vesicles. The state of amphiphiles depends on the concentration. When the concentration is less, amphiphiles introduce a monomer state but as the concentration is increased, variety of structures may be formed, that is, round, cylindrical, disc, cubic, or hexagon type [10].

CHARACTERIZATION OF PHYTOSOMES

Physical attributes

The following are the characterization techniques used for Phytosomes [11]:

a) **Visualization:** Visualization of phytosomes can be done using transmission electron microscopy (TEM) which provides details about internal composition such as morphology, crystallization, stress or even magnetic domains. Scanning electron microscopy (SEM) reveals the surface and its composition and provides morphological details of the phytosomes.

b) **Particle size and zeta potential:** The particle size and zeta potential can be determined by dynamic light

scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).

c) **Entrapment efficiency:** The entrapment efficiency is determined by the ultracentrifugation technique. It gives an idea about the % drug that is successfully entrapped inside the phytosomes.

d) **Transition temperature:** The transition temperature of the phytosomes is determined by differential scanning calorimetry (DSC).

e) **Surface tension activity measurement:** The surface tension activity of the drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

f) **Vesicle stability:** The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. The mean size is measured by DLS and structural changes are monitored by TEM.

g) **Drug content:** The drug content can be estimated by modified high performance liquid chromatographic method or by a suitable spectroscopic method.

Spectroscopic Evaluation

The spectroscopic evaluations are conducted to confirm the formation of complex between phytoconstituents and the phospholipids moiety and also to study the corresponding interaction between the two [4].

a) **^{1}H-NMR:** Using this method, the complex formation between the active phytoconstituents and the phosphatidylcholine molecule can be estimated.

b) **^{13}C-NMR:** Using this method, the phytoconstituents and the stoichiometric complex with the phosphatidylcholine when recorded the phytoconstituents carbons were invisible. The signals corresponding to the glycerol and choline portion are broadened and some are shifted, while most of the resonance of the fatty acid chains retains their original sharp line shape.

c) **FTIR:** The formation of the complex can be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures.

In-Vitro and In-Vivo Evaluations

Models of in-vitro and in-vivo evaluations are selected on the basis of the expected therapeutic activity of the biologically active phytoconstituents present in the phytosomes [12].

APPLICATIONS OF PHYTOSOME AND COMMERCIALLY AVAILABLE PHYTOSOME TECHNOLOGY

Silymarin phytosomes

Most of the Phytosomal studies are concentrated on *Silybummarianum* (milk thistles) which carriesexclusive liver protectant flavonoids. Yanyu [13] formulated silymarin phytosome and evaluated its pharmacokinetic activity in rats. In the experiment, the bioavailability of silybin in rat was increased significantly after oral administration of silybin-phospholipid complex due to magnificent improvement of the lipophilic properties of silybin-phospholipid complex and improvement of biological effect of silybin. Tedesco et al (2004) reported Silymarin phytosome exhibit better anti-hepatotoxic activity than silymarin alone and can provide protection against the toxic effects of aflatoxin B1 on performance of broiler chicks [13].

Phytosomes of *gingko biloba* leaves

Studies have revealed that gingko phytosomes (prepared from standardized extract of *Ginkgo biloba*leaves) exhibited better results compared to the conventional standardized extract from plant (GBE, 24% ginkgo flavones glycoside and 6% terpenes lactones). In a bioavailability study conducted with healthy human volunteers the level of GBE constituents (flavonoids and terpenes) from the Phytosomal form peaked after 3 hours and sustained longer for at least 5 hours after oral administration. It was observed that the Phytosomal GBE produced a 2-4 times higher plasma concentration of terpenes than the non-Phytosomal GBE. Its major indication is cerebral insufficiency and peripheral vascular disorders and it can also ameliorate reduced cerebral circulations. Its improved oral bioavailability and high tolerability makes it the ideal gingko formulation even for long term treatment. Studies have also showed the improved efficacy of ginkgo phytosomes over the conventional standardized extract in protecting rat isolated hearts against ischemia [14].

Curcumin phytosomes

Maiti [14] formulated the phytosomes of curcumin (flavonoid from *Curcuma longa*, turmeric) and naringenin (flavonoid from grape fruit, *Vitisvinifera*) in two different studies. The antioxidant activity of the complex was markedly greater than pure curcumin in all dose level tested. In the second study, the formulated phytosome of naringenin exhibited better antioxidant activity than the free compound with a sustained duration of action, which may be due to decrease in the rapid elimination of the molecule from body [14].

Phytosomes of grape seed extract

Phytosomes of grape seed extract is made of oligomeric polyphenols (grape proanthocyanidins or Procyanidin from grape seed extract, *Vitisvinifera*) of different molecular sizes complexed with phospholipids. The main properties of Procyanidin flavonoids of grape seed include high total antioxidant capacity and stimulation of immune response of plasma, protection from ischemia/reperfusion induced

damage in the heart, protective effects against atherosclerosis hence displaying enhanced protection for the cardiovascular system and other organs through a network of mechanism that extend beyond their antioxidant effect [15].

Phytosome of olive (*Olea Europaea*) oil

A commercially available phytosome- Oleaselect phytosome is present in the market based on olive oil polyphenols [16]. It is a strong antioxidant, anti-inflammatory and anti-hyper-lipidemic. It inhibits the oxidation of LDL cholesterol and is cardioprotective.

Phytosomes of green tea

Green tea leaves (*Theasinensis*) are famous for the presence of a polyphenolic compound epigallocatechin 3-O-gallate as the key compound. These compounds are potent modulators of various biochemical processes linked to the breakdown of homeostasis in serious chronic-degenerative diseases such as cancer and atherosclerosis. Green tea exhibits various beneficial activities such as antioxidant, anticarcinogenic, antimutagenic, hypocholesterolemic, and cardioprotective effects [17].

Quercetin-phospholipid phytosomal complex

Maiti [14] formulated the quercetin-phospholipid Phytosomal complex by a simple and reproducible method and also claimed that the formulation exhibited better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride [17].

Some Patented Technologies Related to Phytosomes

Following are the innovative processes and formulation research studies in the field of phytosomes carried out by a number of academic scientists as well as by industrial laboratories. Some patents for phytosomes and other related technologies along with their applications and innovations are listed in **Table 1** [8].

Table 1. Some patented technologies related to phytosomes.

Title of patent	Innovation	Patent No.
Phospholipid complexes of olive fruits or leaves extracts having improved bioavailability	Phospholipids complexes of olive fruits or leaves extracts or compositions containing it having improved bioavailability	EP/1844785
Compositions comprising Gingko biloba derivatives for the treatment of asthmatic and allergic conditions	Compositions containing fractions deriving from Ginkgo biloba, useful for the treatment of asthmatic and allergic conditions	EP1813280
Fatty acid monoesters of sorbityl furfural and compositions for cosmetic and dermatological use	Fatty acid monoesters of sorbityl furfural selected from two diff series of compounds in which side chain is a linear or branched C3-C19 alkyl radical optionally containing at least one ethylenic unsaturation	EP1690862
Cosmetic and dermatological composition for the treatment of aging or photodamaged skin	Composition for topical treatment of the skin comprises a substance that stimulates collagen synthesis and a substance that enhances the interaction between extracellular matrix and fibroblasts Cosmetic or dermatological composition for topical treatment	EP1640041
Treatment of skin, and wound repair, with thymosin beta 4	Compositions and methods for treatment of skin utilizing thymosin β4.	US/2007/ 0015698
Soluble isoflavone compositions	Isoflavone compositions exhibiting improved solubility (e.g., light transmittance), taste, color, and texture characteristics, and methods for making the same.	WO/2004/ 045541
An anti-oxidant preparation based on plant extracts for the treatment of circulation and adiposity problems	Preparation based on plant extracts which has an anti-oxidant effect and is particularly useful in treatment of circulation problems such as phlebitis, varicose veins, arteriosclerosis, hemorrhoids and high blood pressure	EP1214084
Complexes of saponins with phospholipids and pharmaceutical and cosmetic compositions Containing them	Complexes of saponins with natural or synthetic phospholipids have high lipophilia and improved bioavailability and are suitable for use as active principle in pharmaceutical, dermatologic and cosmetic compositions	EP0283713

Cost-Benefit Analysis of Phytosome Technology

Aside from the modern developments in industrial-scale production of vesicular systems, comprising extruding methods, which offers hopeful prospective for the commercial production of these vesicular systems, the high cost of raw materials viz., PEGylated soy phosphatidylcholine may endanger this advancement. In spite of the endangering limitations ushering the formulation of phytosomes, this concept and technology bears a promising potential in its applications. In the forthcoming future, phytosomal delivery systems will stand to advance and grow for clinical therapy. It is obvious that the engineering of herbal and synthetic agents into phytosomal complexes can remarkably enhance oral bioavailability. Although there are some limitations in the manufacturing process, phytosomes have sufficient advantages to qualify for industrial production (at the pharmaceutical and food industrials) for eliciting therapeutic effects along with developing medicinal-functional foods **(Table 2)** [18].

Table 2. Some commercial phytosome preparations [19].

Phytosomes Phosphatidyicholine	Phytoconstituent complexed	Indication	Dose
Silybin Phytosome	Silybin from silymarin marium	Nutraceutical, antioxidant for Liver and skin	120mg
Ginkgo Phytosome	24% ginkgo flavonoids from *Ginkgo biloba*	Protect brain and vascular lining	120mg
Olive oil Phytosome	Polyphenols from Europaea oil	Antioxidant, anti inflammatory, Anti-hyper lipidemic	-
Grape seed phytosome	Procynid ins from *Vitisvinifera*	Nutraceutical, systemic antioxidant	50-100mg
Haw thorn Phytosome	Flavonoids from carteagus sp.	Nutraceutical, cardio protective, Anti hypertensive	100mg
Centella Phytosome	Terpenes	Vein and skin disorders	-
Ecdhinacea Phytosome	Echinacoside from *Echinacea augustifolia*	Nutraceutical, immune modulator	-

CONCLUSION

The absorption of phytosomes in gastro intestinal tract is substantially higher resulting in the increased plasma level of the phytoconstituent than the conventional formulation. Complex formation ratio of component and phospholipids is 1:1 and 2:1. Phytosomes are widely used as a medicament and have wide scope in cosmeticology. A wide area of phytosome technology is yet to be revealed in future in the prospect of various pharmaceutical applications. Phytosomes forms an appreciable bridge between the conventional delivery systems and novel delivery systems.

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REFERENCES

1. Azeez NA, Deepa VS, Sivapriya V (2018) Phytosomes: Emergent promising nano vesicular drug delivery system for targeted tumor therapy. Adv Nat Sci: Nanosci Nanotechnol 9: 033001.
2. Shivanand P, Kinjal P (2010) Phytosomes: Technical revolution in phytomedicine. Int J PharmTech Res 2: 627-631.
3. Gao L, Liu G, Wang X, Liu F, Xu Y, et al. (2011) Preparation of a chemically stable quercetin formulation using nanosuspension technology. Int J Pharm 404: 231-237.
4. Pawar HA, Bhangale BD, Deshpande PK (2016) Phytosomes : A Noval Drug Delivery System for Phytoconstituents Phytosomes : A Noval Drug Delivery System for Phytoconstituents. J Chem Pharm Res 7: 144-160.
5. Manglani N, Shilpa V (2012) Phytosomes : A Novel Herbal Drug Delivery System. J Pharm Sci Innov 1: 35-40.
6. Thurapati PR, Reddy MS, Veerareddy PR (2011) Phyto-complexs, a novel phyto-phospholipid carriers for herbal drug delivery. Int Res J Pharm 2: 28-33.

7. Singh RP, Narke R (2015) Preparation and Evaluation of Phytosome of Lawsone. Int J Pharm Sci Res 6: 5217-5226.

8. Saha S, Sarma A, Saikia P, Chakrabarty T (2013) Phytosome : A Brief Overview. Sch Acad J Pharm 2: 12-20.

9. Rathore P, Swami S (2012) Planterosomes: A potential phyto-phospholipid carriers for the bioavailability enhancement of herbal extracts. Int J Pharm Sci Res 3: 737-755.

10. Saini V, Rani B, Nagpal M, Arora S (2013) Phytosomes: Potential carriers for herbal drugs. Am J PharmTech Res 3: 250-260.

11. Changediya V, Khadke M, Devdhe S (2011) Phytosomes: New approach for delivering herbal drug with improved bioavailability. Res J Pharm Biol Chem Sci 2: 57-68.

12. Awasthi R, Kulkarni GT, Pawar VK (2011) Phytosomes: An approach to increase the bioavailability of plant extracts. Int J Pharm Pharm Sci 3: 1-3.

13. Yanyu X, Yunmei S, Zhipeng C, Qineng P (2006) The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int J Pharm 307: 77-82.

14. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK (2007) Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm 330: 155-163.

15. Kidd PM (2009) Bioavailability and activity of phytosome complexes from botanical polyphenols: The silymarin, curcumin, green tea, and grape seed extracts. Alt Med Rev 14: 226-246.

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

17. Maiti K, Mukherjee K, Gantait A, Ahamed HN, Saha BP, et al. (2005) Enhanced therapeutic benefit of quercetin– phospholipid complex in carbon tetrachloride-induced acute liver injury in rats: A comparative study. Iran J Pharmacol Ther Res 405: 1735-2657.

18. Babazadeh A, Jafari SM, Shi B (2019) Encapsulation of food ingredients by nanophytosomes: Lipid-Based Nanostructures for Food Encapsulation Purposes 2: 405-443.

19. Sravanthi M, Krishna JS (2013) Phytosomes: A Novel Drug Delivery for Herbal Extracts. Int J Pharm Sci Res 4: 949-959.