PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

PHYTOSOMES: NOVEL APPROACH FOR DELIVERING HERBAL EXTRACT WITH IMPROVED BIOAVAILABILITY

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ABSTRACT

Plant-derived polyphenols are increasingly gaining attention as dietary supplements for their multidimensional role in homeostatic management of inflammation, to support detoxication, and for anticancer, weight loss, and many other benefits. Many plants extracts rich in flavonoids and polyphenolics have been extensively explored for their pro-homeostatic effects on genes, transcription factors, enzymes, and cell signaling pathways, but the poor bioavailability of some polyphenols likely contributes to poor clinical trial outcomes. Bioavailability of flavonoids, both in aglyconic or glycosidic form, is reported to be low and erratic due to limited absorption, elevated presystemic metabolism and rapid elimination. The phytosome technology creates intermolecular bonding between individual polyphenol molecules and one or more molecules of the phospholipids, phosphatidylcholine (PC). A Phytosome is generally more bioavailable than a simple herbal extract due to its enhanced capacity to cross the lipid-rich biomembranes and reach circulation. As a molecular delivery vehicle, phytosome technology substantially enhanced the bioavailability of many popular herbal extracts including milk thistle, Ginkgo biloba, grape seed, green tea, hawthorn, ginseng etc and can be further developed for clinical applicability of polyphenols and other poorly absorbed phytoconstituents. This article reviews the recent advances and applications of various standardized herbal extract phytosomes as a tool of drug delivery.

Keywords: Herbal extracts; Bioavailability; Phytosomes; Polyphenolics; Herbal Drug delivery; Phospholipid complex.

INTRODUCTION

Herbal medicine is a practice as old as mankind and during the last century chemical and pharmacological studies have been performed on a lot of plant extracts in order to know their chemical composition and to confirm the indications of traditional medicine. Phytochemical and phytopharmacological studies have long been established for the compositions, therapeutics and overall health boosting capacities of various plant

products but there is a great interest and medical need for the improvement of bioavailability of a large number of herbal drugs and plant extracts which are poorly lipid soluble and so less bioavailable. [1] Many herbal drugs or herbal extracts despite of their extraordinary potential *invitro* finding demonstrate less or no *in vivo* actions due to their poor lipid solubility or improper molecular size or both, ultimately resulting in poor absorption and poor bioavailability. Various components of an extract may contribute to the synergic action of the extract and treatment like purification and separation can lead to a partial loss of specific activity due to the removal of chemically related substances contributing to the activity of the main components. Very often the chemical complexity of the extract seems to be important for the bioavailability of the active components. Most of the plant constituents specifically phenolics are water soluble and so the major problem for less bioavailability is the inability to cross the lipid membranes of intestine. The bioavailability can be improved with the use of different novel delivery systems like liposomes, marinosomes, niosomes and photosomes which can enhance the rate of release as well as the capacity to cross the lipid rich biomembranes. [2,3] Phospholipids based drug delivery systems have been found much hopeful and promising for the effective and efficacious herbal drug delivery. Phytosomes are advanced forms of herbal products that are better absorbed, utilized, and as a result produce better results than the conventional herbal extracts. Water-soluble phytoconstituents like many polyphenolics and flavanoids can be converted into a lipid-compatible molecular complex with help of this technology to impart the herbal extract enhanced capacity to cross the lipid-rich biomembranes and reach circulation. Phytosomes are produced via a patented process whereby the individual components of an herbal extract are bound to specific phospholipids [4-7]. Phospholipids are small lipid molecules where glycerol is bonded to two fatty acids, while the third hydroxyl, normally one of the two primary methylenes, bears a phosphate group bound to a biogenic amino or to an amino acid. Phospholipids are a class of lipids and are a major component of all cell membrane. In humans and other higher animals the phospholipids are also employed as natural digestive aids and as carriers for both fat-miscible and water miscible nutrients. They are miscible both in water and in lipid environments, and are well absorbed orally. Phospholipids from soybean, *(Glycine max)* mainly phosphatidylcholine is a lipophilic agent that readily

complex polyphenolics and widely employed to make phytosomes.[8] Phosphatidylcholine, the major molecular building block of cell membranes is a compound miscible in both water and in oil/lipid environments. [9] The phytosomes has more ability to carry the herbal extract of hydrophilic nature through the lipid bilayer and thus it is more bioavailable compared to simple extract. The phytosome technology has been reported to effectively enhance the bioavailability of many popular herbal extracts including milk thistle, ginkgo biloba, grape seed, green tea, hawthorn, ginseng turmeric, centella, ammi etc and can further be developed for various therapeutic uses or dietary supplements. [10,11]

Phosphatidylcholine and Herbal Extract

Phospholipids are a class of lipids and are a major component of all cell membrane. In humans and other higher animals the phospholipids are also employed as natural digestive aids and as carriers for both fat-miscible and water miscible nutrients. They are a major component of biological membrane and can be isolated from either egg yolk or soy beans from which they are mechanically extracted or chemically extracted using hexane. Phosphatidylcholine is a bifunctional compound, the phosphatidyl moiety being lipophilic and the choline moiety being hydrophilic in nature. Specifically the choline head of the phosphatidylcholine molecule binds to the components of herbal extract while the lipid soluble phosphatidyl portion then envelopes the choline bound material that results in a little micro sphere or cell is produced. The term "phyto" means plant while "some" means cell-like. What the Phytosome process produces is a microsphere cell that protects valuable components of the herbal extract from destruction by digestive secretions and gut bacteria. [10,11]

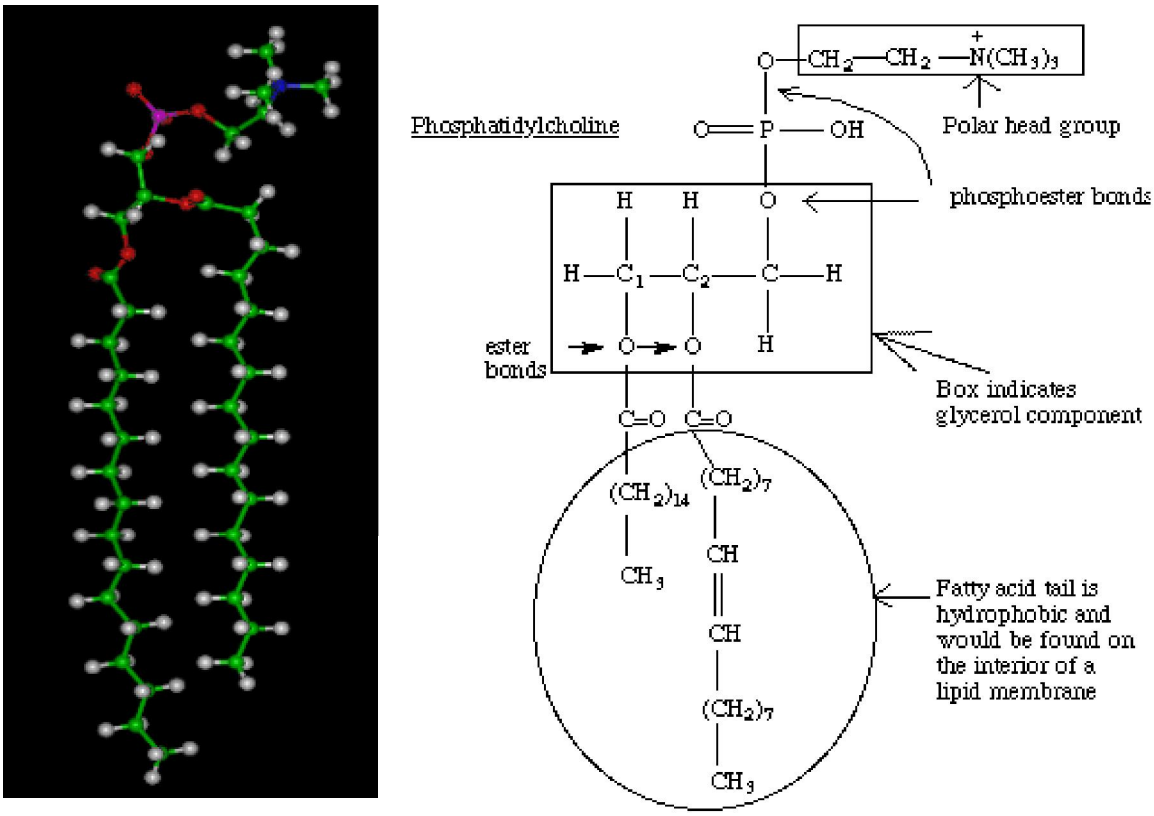

Figure 1
Phosphotidylcholine orientation.

Many popular standardized herbal extracts comprising of flavanoids, polyphenolics, terpenes, alkaloids, volatile oils are employed for the preparation of phytosomes. The hypothesis of an interaction with phospholipids originated from a histochemical finding indicating that anthocyanosides from *Vaccinium myrtillus L.* showed a strong affinity for specific cellular structures rich in phospholipids. Evidence that flavonoids as well as saponins and triterpenic acids, do form real complexes with phospholipids was obtained about years ago when these complexes could be prepared and chemically standardized.

Mainly flavanoids and polyphenolics are complexed with the phospholipids molecules and forms phytosomes. More than 4,000 naturally occurring flavonoids have been identified, each with its own distinctive molecular structure and 3-D shape. Flavonoids are part of a broader class of dietary antioxidants called polyphenols and are distinctive for their triple ring structures. [10,12,13]

The poor absorption of polyphenolics is likely due to two main factors. First, these are multiple-ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion nor does the intestinal lining actively absorb them, as occurs with some vitamins and minerals. Second, they typically have poor miscibility with oils and other lipids. This severely limits their ability to pass across the lipid-rich outer membranes of the enterocytes, the cells that line the small intestine. PC is miscible both in the water phase and in oil/lipid phases, and is excellently absorbed when taken by mouth. The molecular properties of PC and precise chemical analysis indicate the unit phytosome is usually a herbal extract molecule linked with at least one PC molecule. A bond is formed between the two molecules to create a hybrid molecule. This hybrid is highly lipid - miscible, better suited to merge into the lipid phase of the enterocyte's outer cell membrane. Once there, it can cross the enterocyte and reach the circulating blood.

Preparation of Phytosome

Phytosomes are prepared by reacting from 3-2 moles but preferably with one mole of natural or synthetic phospholipids, with one mole of component like flavolignans, either alone or in the natural mixture in aprotic solvent such as- dioxane or acetone. The phytosome complex can be then isolated by precipitation with non solvent such as aliphatic hydrocarbons or lyophilization or by spray drying. In the complex formation of phytosomes the ratio between these two moieties is in the range from 0.5-2.0 moles. The most preferable ratio of phospholipids to flavonoids is 1:1. [14, 15]

Yanyu et al. (2006) prepared silybin-phospholipid complex using ethanol as a reaction medium. Silybin and phospholipids were resolved into the medium, after the organic solvent was removed under vacuum condition, silybinphospholipid complex was formed [14] Marena and Lampertico (1991), Jiang et al. (2001), Maiti et al. (2006) and Maiti et al. (2006) reported the methods of phytosome preparation [16-19].

In the phytosome preparations, phospholipids are selected from the group consisting of soy lecithin, from bovine or swine brain or dermis, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine in which acyl group may be same or different and mostly derived from palmitic, stearic, oleic and linoleic acid. Selection of flavonoids are done from the group consisting of quercetin, kaempferol, quercretin-3,

rhamnoglucoside, quercetin-3-rhamnoside, hyperoside, vitexine, diosmine, 3-rhamnoside, (+) catechin, (-) epicatechin, apigenin-7-glucoside, luteolin, luteolinglucoside, ginkgonetine, isoginkgonetine and bilobetine. [20]

Phytosomes are different than liposomes in the way it incorporates the water soluble drug to form the complex. A liposome is formed by mixing a water soluble substance with phosphatidylcholine in definite ratio under specific conditions. Here, no chemical bond is formed; the phosphatidylcholine molecules surround the water soluble substance. In contrast, the phytosome process the phosphatidylcholine and the plant components actually form a 1:1 or a 2:1 molecular complex depending on the substance(s) complexed, involving chemical bonds. Fundamental differences are that in liposomes, the active principles are dissolved in the central part of the cavity, with no possibility of molecular interaction between the surrounding lipid and a hydrophilic substance. [21,22] On the other hand the phytosome complex can somewhat be compared to an integral part of the lipid membrane, where the polar functionalities of the lipophilic guest interact via hydrogen bonds with the polar head of a phospholipids (i.e. phosphate and ammonium groups), forming a unique pattern which can be characterized by Spectroscopy. [23,24,25] This difference results in phytosome being much better absorbed than liposomes showing better bioavailability. Phytosomes are also superior to liposomes in skin care products while the liposome is an aggregate of many phospholipids molecules that can enclose other phytoactive molecules but without specifically bonding to them. Liposomes are touted delivery vehicles, but for dietary supplements their promise has not been fulfilled. But for phytosome products numerous studies prove they are markedly better absorbed and have substantially greater clinical efficacy. Companies have successfully applied this technology to a number of standardized flavonoids preparations.

Some liposomal drugs complex operate in the presence of the water or buffer solution where as phytosomes operate with the solvent having a reduced dielectric constant. Starting material of component like flavonoids is insoluble in chloroform, ethyl ether or benzene. They become extremely soluble in these solvents after forming phytosomes. This chemical and physical property change is due to the formation of a true stable complex. [20]

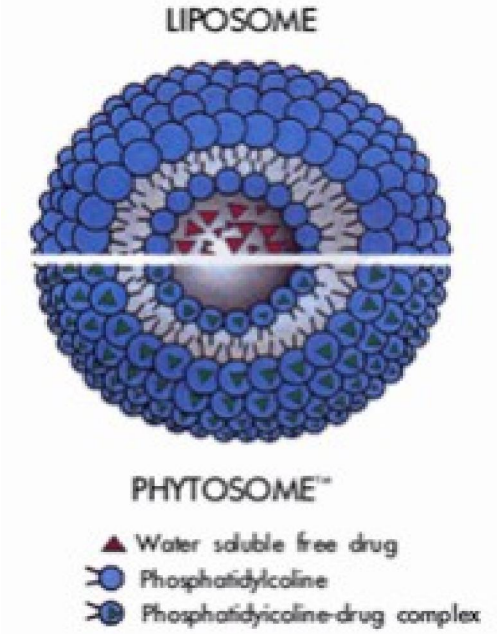

Figure 2
Structural difference between phytosome and liposome.

Characterization of Phytosome

The behavior of phytosomes in both physical and biological system is governed by the factors such as physical size, membrane permeability; percentage entrapped solutes, chemical composition, quantity and purity of the starting materials.

Therefore, the phytosomes are characterized for physical attributes like shape, size, distribution, percentage drug capture entrapped volume, percentage drug released and chemical composition. [21] Complexation and molecular interactions between phytoconstituents and phosphatidylcholine in solution have been studied by ^{1}H-NMR [23-26], ^{13}C-NMR, [4, 24, 26-29] ^{31}P-NMR, [24, 25, 26] as well as by IR spectroscopy [23]. Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) are other techniques employed for the detection and measurement of thermal effects such as fusion, solid–solid transitions, glass transitions, loss of solvent, and decomposition to characterize a solid phytosome. [30] Further NMR data available on the marketed phytosomes also indicates that the signals of the fatty chain are almost unchanged. Such evidences inferred that the two long aliphatic chains are wrapped around the active

principle, producing a lipophilic envelope, which envelope the polar head of the phospholipids and the herbal extract. [31]

Advantages of Phytosome [23, 32, 33]

Phytosomes have following advantages

1) Phytosome are better bioavailable botanical extracts, dramatically enhance bioavailability due to their complex with phospholipids and delivers faster and improved absorption in intestinal tract
2) They enhance the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability with significantly better therapeutic benefit
3) Dose requirement can be minimized as the bioavailability is increased.
4) Phosphatidylcholine used in preparation of phytosomes besides acting as a carrier also acts as a hepatoprotective substance showing the synergistic effect when hepatoprotective substances like flavanoids are employed to form complex.
5) Phytosome are widely used in cosmetics due to there more skin penetration and high lipid profile.
6) Phytosomes show better stability profile owing to the chemical bonds formed between phosphatidylcholine molecule and phytoconstituents.

Improved Bioavailability of Herbal Extract with Improved Results

The phytosome process has been applied to many popular herbal extracts including *Ginkgo biloba, grape seed, hawthorn, milk thistle, green tea,* and *ginseng* and recent research shows improved absorption and bioavailability with phytosomes as compared to the conventional means. Many standardized extract containing flavanoids and polyphenolics have been reported with improved bioavailability when incorporated in photosomal preparation. Silymarin is some of the most studied drug for the better delivery of silybin by forming silybinphospholipid complex. Yanyu et al. prepared the silymarin phytosome and studied its pharmacokinetics in rats. In the study the bioavailability of silybin in rats was increased remarkably after oral administration due to an improvement of the lipophilic property of silybin-phospholipid complex [14]

TABLE 1 COMMERCIALLY AVAILABLE PHYTOSOME PREPARATIONS

[10,11,34]

Sr.no	Phytosome	Phytoconstituents complexed	Indications
1	Silybin Phytosome	Silybin from Milk thistle seed	Hepato –Protective, antioxidant,
2	Ginkgo Phytosome	Flavanoids from *Ginkgo biloba*	Anti-ageing, Protects brain and vascular lining
3	Ginseng Phytosome	Ginsenosides from *Panax ginseng*	Immunomodulator
4	Green Tea Phytosome	Epigallocatechin from *Thea sinesis*	Systemic Antioxidant Anticancer
5	Grape Seed Phytosome	Procyanidins from *Vitis vinifera*	Cardio-Protective Systemic Antioxidant
6	Hawthorn Phytosome	Flavanoids from Crataegus species	Anti Hypertensive Cardio-Protective
7	Olive Oil Phytosome	Polyphenols from *Olea europea*	Anti-Inflammatory Anti-Hyperlipidemic
8	Echinacea Phytosome	Echinacosides from *Echinacea angustifolia*	Immunomodulator, nutraceuticals
9	Sericoside Phytosome	Sericosides from *Terminalia sericea*	Skin Improver
10	Visnadine Phytosome	Visnadine from *Ammi visnaga*	Circulation Improver
11	Centella phytosome	Terpenes from *Centella asiatica*	Vein and skin disorders
12	Palmetto berries phytosomes	Fatty acids, alcohols and sterols	Non cancerous prostate enlargement, antioxidant
13	Bilberry phytosomes	Anthocyanosides extract	Antioxidants, improvement of capillary tone

Tedesco et al. (2004) reported Silymarin phytosome with better anti-hepatotoxic activity than Silymarin alone and can provide protection against the toxic effects of aflatoxin B1 on performance of broiler chicks. [35]

Busby et al. (2002) reported that the use of a silymarin phytosome showed a better fetoprotectant activity from ethanol-induced behavioral deficits than uncomplexed silymarin. [36]

Grange et al. (1999) conducted a Series of studies on silymarin phytosome, containing a standardized extract from the seeds of *S. marianum*, administered orally and reported protective effect on the fetus from maternally ingested ethanol. [37]

Bombardelli et al. (1991) reported Silymarin phytosomes showed much higher specific activity and a longer lasting action than the single constituents, with respect to percent reduction of odema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging properties [33]. Barzaghi et al. (1990) conducted a human study

designed to assess the absorption of silybin when directly bound to phosphatidylcholine. Plasma silybin levels were determined after administration of single oral doses of silybin phytosome and a similar amount of silybin from milk thistle in healthy volunteers. The results indicated that the absorption of silybin from silybin phytosome is approximately seven times greater compared to the absorption of silybin from regular milk thistle extract (70-80 % silymarin content) [38].

Mascarella et al. (1993) investigated in one study of 232 patients with chronic hepatitis (viral, alcohol or drug induced) treated with silybin phytosome at a dose of 120 mg either twice daily or thrice daily for up to 120 days. Liver function returned to normal faster in patients taking silybin phytosome compared to a group of controls where 49 patients treated with commercially available silymarin and 117 untreated or given placebo. [39]

Studies have shown ginkgo phytosome (prepared from the standardized extract of Ginkgo biloba leaves) produced better results compared to the conventional standardized extract from the plant (GBE, 24% ginkgo flavone glycoside and 6 % terpene lactones). In a bioavailability study conducted with healthy human volunteers, it was found that the phytosomal GBE produced a 2-4 times greater plasma concentration of terpenes than did the non-phytosomal GBE. Its major indications are cerebral insufficiency and peripheral vascular disorders, and it also can ameliorate reduced cerebral circulation. In studies with ginkgo phytosome in patients with peripheral vascular disease (e.g. Raynaud's disease and intermittent circulation) it was shown to produce a 30-60 % greater improvement compared to regular standardized GBE. [11,40,41] Ginseng saponin phytosome possesses a transdermal action demonstrated by the objective improvement in the cutaneous elasticity and tone, which could be related to increased blood perfusion with dilatation of capillaries and arterioles, leading to improved delivery of nutrients to the skin compared to normal extract. [26]

Grape seed phytosome is composed of oligomeric polyphenols (grape proanthocyanidins or procyanidins from grape seed extract, *Vitis vinifera*) of varying molecular size, complexed with soy phospholipids (1:3 w/w). The main properties of procyanidin flavonoids of grape seed are an increase in total antioxidant capacity and stimulation of physiological antioxidant defenses of plasma, protection against

ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis thereby offering marked protection for the cardiovascular system.

In a study where rabbits were fed a high cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol and induce atherosclerotic lesions in their aortas and carotid arteries, the group that received grape seed phytosome in their feed for the first 6 weeks, then 4 weeks of the high cholesterol diet developed significantly less aortic plaque when compared with the group treated with conventional standardized grape seed extract in similar regimen. Further in a randomized human trial, the healthy volunteer group that received grape seed phytosome, blood TRAP (Total Radical-trapping Antioxidant Parameter) levels were significantly elevated within 30 mins after administration on 1st day over the control which received conventional standardized grape seed. [42]

Green tea extract generally contains a totally standardized polyphenolic fraction mainly characterized by the presence of epigallocatechin 3-O-gallate. Despite potential actions of green tea as antioxidant, anticarcinogenic, antimutagenic, antiatherosclerotic and hypocholesterolemic green tea polyphenols have very poor oral bioavailability from conventional extracts. It was reported that absorption of phytosomal preparations was more in healthy human volunteers following oral administration compared to non complexed green tea extract. Over the study period of 6 hours the plasma concentration of total flavonoids was more than doubled and antioxidant capacity measured as TRAP showed 20% enhancement with the phytosome formulation when compared to the conventional standardized extract. [40,43]

Further clinical trail studies on caffeine free green tea phytosomes showed significant antiobesity and antioxidant activities. It was also reported to possess lipid lowering effect particularly on LDL. [44, 45]

Maiti et al. (2005) developed the quercetin-phospholipids phytosomal complex that exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride [46] Further Maiti et al. (2006) developed the phytosomes of curcumin and naringenin in two different studies with significantly higher antioxidant effect with prolonged duration of action than the pure or umcomplexed compound [18,19]

Marczylo et al. 2007 reported the compared bioavailability of standardized curcumin extract, pure curcumin and curcumin phytosomes when evaluated in rats, and revealed

that phytosomal preparation was found five times more bioavailable compared to pure compound and extract. [47]

Further phytosome formation can also reduce side effects of the pure compound or herbal extract as in case of ecsin and β-sitosterol phytosome reported. The major indication of ecsin is mainly related to the modification of vascular permeability and used widely in cosmetics. Phytosome show comparatively less irritation on the skin compare to the extract itself when evaluated for irritation on rabbit skin. [28]

18ß-glycyrrhetinic acid phytosome is the complexed form of 18ß-Glycyrrhetinic Acid with soy phospholipids**.** The passage of the compound through the skin takes place through the interaction with cutaneous structures. In the reticular layer of the dermis, the complex is thought to undergo a slow and progressive decomplexation resulting in the in situ release of the free active constituent. The active component 18ß-Glycyhrretinic Acid is structurally similar to cortisol, and potentiates the anti-inflammatory activity of cortisol by inhibiting its intracellular inactivation. [27]

Recently much amount of work is going on various new standardized herbal extract to formulate into more bioavailable phytosomes. Extract of *Serenoa repens* (CO_2 extract) extract of *Viccinium myrtillus* (Fruit extract), extract of *Colues forskohlii*, Ximenoil and Ximenynic acid extracted from *Santalum album,* Esculoside, glycosidated coumarin obtained form *Aesculus hippocastanum*, Ruscogenins, group of saponins extracted from *Ruscus aculeatus* are highly worked upon for better bioavailability through the formation of phytosomes by patented process.[40]

CONCLUSION

A Phytosome is a complex between polar polyphenolics and dietary phospholipids that shows definite physicochemical and spectroscopic features. Recent technology of drug delivery when applied to botanicals open new avenues to explore maximum therapeutic potential of plant substances of polar nature. Phytosomal complexes were first investigated for cosmetic applications, but mounting evidence of potential for drug delivery has been cumulated over the past few years, with beneficial activity in the realms of cardiovascular, anti-inflammatory, hepatoprotective and anticancer applications. Standardized plant extracts or mainly polar phytoconstituents like flavonoids, terpenoids, tannins, xanthones when complexed with phospholipids like

phosphatidylcholine give rise to a new drug delivery technology called phytosome showing much better absorption profile following oral administration owing to improved lipid solubility which enables them to cross the biological membrane, resulting enhanced bioavailability. Phytosomes have improved pharmacokinetic and pharmacological parameter, which in result can advantageously be used in treatment of various acute diseases as more amount of active constituent becomes present at the site of action (liver, brain, heart, kidney etc) at similar or less dose as compared to the conventional plant extract.

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