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PLANTEROSOMES: A POTENTIAL PHYTO-PHOSPHOLIPID CARRIERS FOR THE BIOAVAILABILITY ENHANCEMENT OF HERBAL EXTRACTS

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ABSTRACT

Planterosomes, term "PLANTERO" means plant while "SOME" means cell-like. A novel emerging technique applied to phytopharmaceutical for the enhancement of bioavailability of herbal extract for medicinal applications. Since the two major limiting factors for molecules to pass the biological membrane for their absorption in the blood stream mainly includes lipid solubility and molecular sizes. There are many plant extracts having excellent bioactivity in vitro but low or less in vivo because of their poor lipid solubility and improper size of the molecule or both which result in poor absorption and bioavailability of constituents from plant extract and are destroyed in the gastric fluids when taken orally. Planterosomes are recent advanced forms of herbal formulations that have enhanced absorption rate, producing better bioavailability than conventional herbal extracts. Since they have improved pharmacological and pharmacokinetic parameters, they can be used in the treatment of the acute and chronic liver disease. Planterosomes are prepared by non conventional methods. Planterosomes absorption in GIT is greater resulting in increased plasma level than individual component. They act as a bridge between novel delivery system and conventional delivery system. Phospholipids molecule acting as vital carrier made up of water soluble head and two fat soluble tails, due to this nature they possess dual solubility and thus acting as an effective emulsifier. These drug-phospholipids complex can be formulated in the form of solutions, suspensions, emulsions, syrup, lotion, gel, cream, aqueous microdispersions, pill, capsule, powder, granules and chewable tablets. Planterosomes just like Phytosomes technology effectively enhanced the bioavailability of many popular herbal extracts including milk thistle, *Ginkgo biloba*, grape seed and ginseng etc.

INTRODUCTION: Phytomedicines, complex chemical mixture prepared from plants, have been used in medicine since ancient times and continue to have widespread popular use. The use of Planterosomes is a new advanced modern dosage formulation technology to deliver herbal products and drugs with improved better absorption and, as a result, produce better result than those obtained by conventional herbal extract [1, 2]. Most of the bioactive constituents of phytomedicines are water-soluble molecules (e.g., phenolics, glycosides and flavonoids). However, water soluble phytoconstituents are limited in their effectiveness because they are poorly absorbed [3] when taken orally or when applied topically.

Many approaches have been developed to improve the oral bioavailability, such as inclusion by solubility and bioavailability enhancers, structural modification and entrapment with the lipophilic carriers [4-6]. There are many factors which may contribute to the poor bioavailability. For example, many phytoconstituents have multiple rings and therefore, cannot be absorbed from the intestine into the blood by simple diffusion.

Also, some herbal phytomolecules are poorly miscible with oils and other lipids and often fail to pass through the small intestine because of its lipoidal nature. Plants are endowed with a multitude of medicinal and health giving substances, most of them are secondary metabolite, prominent among these being the flavonoids.

First recognized for their antioxidant properties, flavonoid is widely distributed in plants. To date, more than 4,000 naturally occurring flavonoid have been identified from plant source having diverse biological activities [7]. The hypothesis of an interaction of flavonoid with phospholipids, which are ubiquitous in plant and animals, originated from the histochemical finding indicating that anthocyanosides from *Vaccinium myrtillus* L. show a strong affinity for specific cellular structure rich in phospholipids [8].

The effectiveness of any herbal product is dependent upon delivering an effective level of the active compounds. Advanced biochemical and pre-clinical studies have proved the potential of plant flavonoids and other hydrophilic natural compounds for the treatment of skin disorders, different types of carcinoma, anti-aging and many other areas of therapeutics and preventive medicine. The hydrophilic nature and unique chemical structure of these compounds pose major challenge because of their poor bioavailability through the skin or gut. The use of herbosomes is novel formulation technology which helps to overcome these problems.

Multiple approaches to improve Bioavailability: Drug bioavailability is a well known issue in the pharmaceutical sector [9] and different strategies have been developed to ameliorate the absorption. Also in case of poorly absorbed natural derived ingredients, various strategies are being followed in the nutraceutical sector to achieve this goal.

The first one might also seem to the medicinal chemistry approach: by the chemical derivatization of the chemical product, the aim is to obtain compounds showing an improved bioavailability. This approach, however, generates a number of chemical analogues that need to be appropriately screened. An alternative strategy that is also being pursued is the combination of the active molecules with other compounds as adjuvants promoting the active molecule's absorption [10].

A third approach involves extensive formulation research of structures capable of both stabilizing natural molecules and promoting their intestinal absorption. The formulative research comprises the formation of liposomes, micelles, nanoparticles, nanoemulsions, microsphere or other complexes.

The planterosomes approach has the improved pharmacokinetic profile is obtained without resorting to pharmacological adjuvant or structural modification of the ingredients, but by formulating them with a dietary ingredient (soy lecithin)

Type of 'Somes': Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment, and channel the active entity to the site of action.

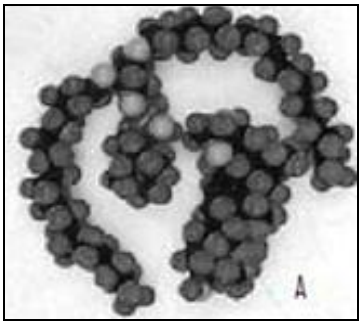
A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved.

Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of systems that allow drug targeting, and the sustained or controlled release of conventional medicines.

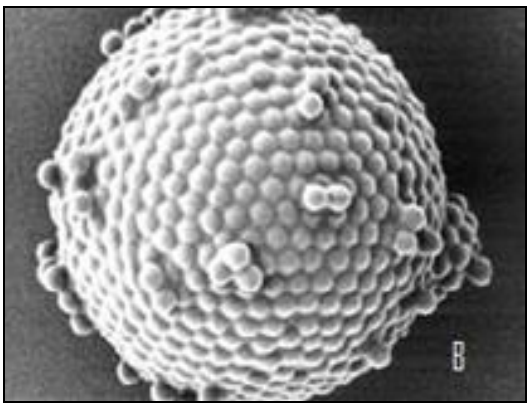
Emerging "Somes" and their Applications [11]

Vesicular system	Description	Application
Enzymosomes	Liposomal construct engineered to provide a mini bio environment in which the enzyme covalently immobilized to the surface of liposomes	Targeted delivery to tumor cell
Virosomes	Liposomes spiked with virus glycoprotein's, incorporated in the liposomal bilayer based on retrovirus based lipids	Immunological adjuvants
Ufasomes	Vesicles enclosed by fatty acids obtained by long chain fatty acids by mechanical agitation of evaporated film in the presence of buffer solution	Ligand mediated drug targeting
Cryptosomes	Lipid vesicle with surface coat composed of PC and of suitable polyoxyethylene derivative of phosphatidyl ethanolamine	Ligand mediated drug delivery
Emulsomes	Nanosized lipid particles consisted of lipid assembly and a polar group	Parenteral delivery of poorly water soluble drugs
Discosomes	Niosomes coupled with non-ionic surfactants	Ligand mediated drug targeting
Aquasomes	These are spherical 60-300 nm particles used for drug and antigen delivery. The particle core is composed of noncrystalline cacium phosphate or ceramic diamond and is covered by a polyhydroxyl oligomeric film	Specific targeting, molecular shielding
Collidosomes	Are solid microcapsules formed by the self assembly of colloidal particles at the interface of emulsion droplets. "Colloidosomes" are hollow, elastic shells whose permeability and elasticity can be precisely controlled.	Drug targeting
Genosomes	Artificial macromolecular complex for functional gene transfer	Cell specific gene transfer
Photosomes	Photolyase encapsulated in liposomes, which release the contents by photo triggered charges in membrane permeability characteristics.	Photodynamic therapy
Erythrosomes	Liposomal system in which chemically cross-linked human erythrocytes cytoskeletons are used as to which a lipid bilayer is coated	Targeting of macromolecular drugs
Hemosomes	Heamoglobin containing liposomes engineered by immobilizing heamoglobin with polymerizable phospholipids	High capacity oxygen carrying system
Protosomes	High molecular weight multisubunit enzyme complexes with catalytic activity	Better catalytic activity turnover than non associated enzymes
Vesosomes	Nested bilayer composed of bilayer	Multiple compartment of vesosomes give better protection to the interior content of serum
Archaeosomes	Vesicles composed of gylcerolipids of archaea with potent adjuvant activity	Poor adjuvant activity
Cubosomes	Are bicontinuous cubic phases, consisting of two separate, continuous, but non intersecting hydrophilic regions divided by a lipid layer that is contorted into a periodic minimal surface with zero average curvature.	Drug targeting

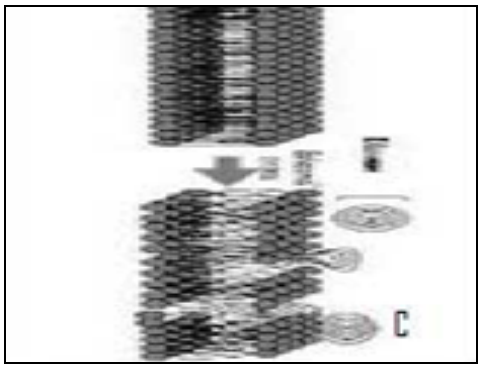
The Wide Spectra of Established 'Somes':



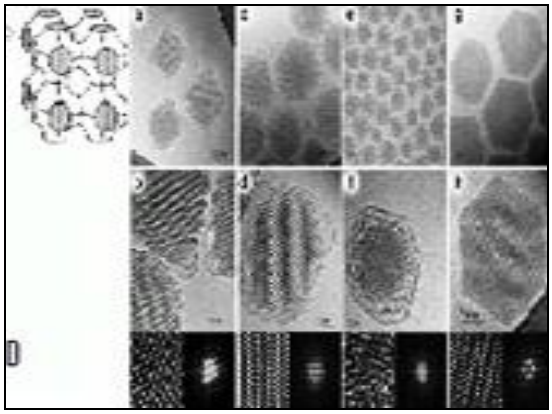
(A) ORGANIZATION OF THE PLANTEROSOME MOLECULAR COMPLEX



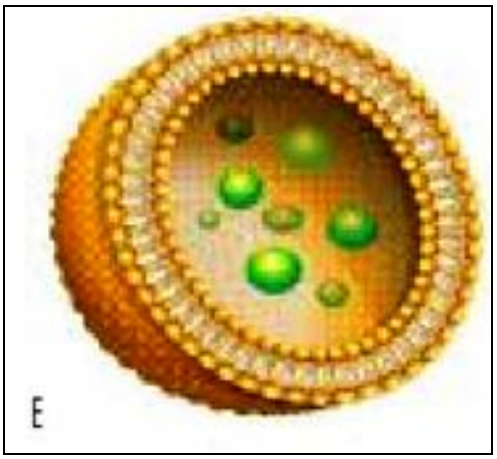
(B) COLLOIDOSOMES



(C) ETHOSOMES



(D) CUBOSOMES



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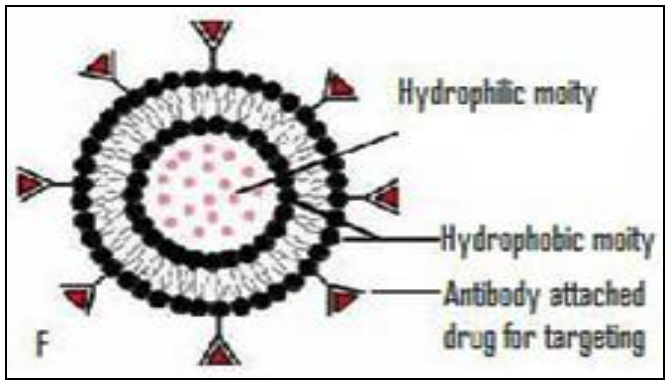
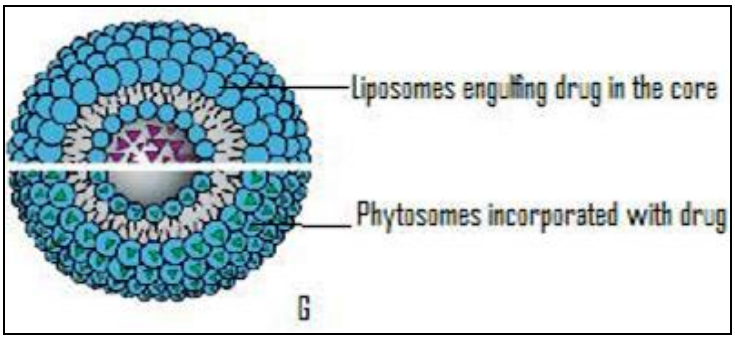


(F) NIOSOMES



(G) DIFFERENTIATION OF PHYTOSOME AND LIPOSOMES

Planterosome: Planterosome are created when the standardized extract and active ingredients of an herb are bound to the phospholipids on a molecular level. Planterosome structures contain the active ingredients of the herb surrounded by the phospholipids.

Planterosome is a patented process developed by Indena, a leading supplier of nutraceutical ingredients, to incorporate phospholipids into standardized extract and so vastly improve their absorption and utilization. Certain of the water-soluble phyto-molecules (mainly flavonoids and other polyphenols) can be converted into lipid-friendly complexes, by reacting herbal extract owing to their enhanced capacity to cross the lipid-rich biomembranes and, finally, reach the blood[12]. They have improved pharmacokinetic and pharmacological parameters which are advantageous in the treatment of acute disease as well as in pharmaceutical and cosmetic compositions [13].

Lipid solubility and molecular size are the major limiting factors for molecules to pass the biological membrane to be absorbed systematically following oral or topical administration. Several plant extracts and phytoconstituents, despite having excellent bio-activity in vitro demonstrate less or no in vivo actions due to their poor lipid solubility or improper molecular size or both, resulting poor absorption and poor bioavailability. Development of herbosomes is at the budding stages in India and abroad.

These drug-phospholipids complexes can be formulated in the form of solution, suspension, emulsion, syrup, lotion, gel, cream, aqueous microdispersion, pill, capsule, powder, granules and chewable tablet phosphatidylcholine resulting in a product that is better absorbed and produces better result than the conventional herbal extracts. Planterosomes also has added dimensions; the proven

health giving activity of the phospholipids themselves. The presence of a surfactant i.e. the phospholipids in the molecule allows obtaining a higher adhesion of the product itself to the surface it comes into contact with and a better interaction of various molecules with cell structure. This aspect is of paramount importance in cosmetics and pharmaceutical formulations.

The Planterosome technology enables cost effective delivery and synergistic benefits from the phospholipids nutraceuticals intrinsic to life. The phospholipids mainly employed to make planterosomes, is phosphatidylcholine, derived from soybean (glycine max). Planterosomes are more bioavailable as compared to conventional herbal extract owing to their enhanced capacity to cross the lipoidal biomembrane and finally reaching the systemic circulation. Planterosome has been an emerging trend in delivery of herbal drugs and nutraceuticals.

Phospholipids:

For the improvement of nutrient absorption and bioavailability: Phospholipids (pronounced fos-fo-lip-ids) are complex substance with chemical, biochemical and nutritional characteristics that place them in a unique nutritional category. They are complex lipid molecules indispensable for life and are abundant in all human and the other known forms to make cell membranes. The profound biochemical importance of phospholipids is reflected in their extraordinary clinical benefits as dietary supplements. The phospholipids are readily compatible with the entire range of vitamins, minerals, metabolites, and herbal preparations currently consumed as the dietary phospholipids and omega-3 fatty acid works in functional synergy in cell membranes.

Phosphatidylcholine is a bifunctional compound miscible both in water and in oil environments, and is well absorbed when taken by mouth. Phosphatidylcholine is not merely a passive “carrier” for the bioactive compounds, but is itself a bioactive nutrient with documented clinical efficacy for liver disease, including alcoholic hepatitis. Phosphatidylcholine is present in egg yolk, brain tissue and a wide variety of animal fat and plant oils. It is routinely present in the bile fluid, to help emulsify food ingredient for absorption.

It work in concert with the body’s orthomolecular antioxidants (vitamin C & E, alpha-lipoic acid, glutathione, coenzyme Q10, selenium, zinc, manganese, copper) to protect membranes in the liver and other organs. Also, when phosphatidylcholine and other phospholipids are taken as dietary supplement along with food are better absorbed [14].

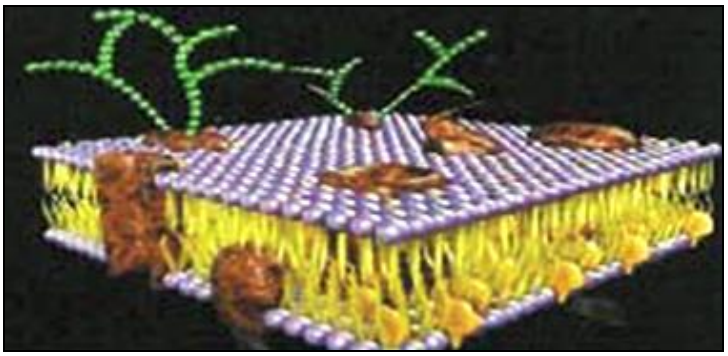


FIG. 1: CELL MEMBRANE WITH HYDROPHILIC AND LIPOPHILIC PHASE

A number of drug delivery system are based entirely on phosphatidylcholine such as liposomes, ethosomes,phytosomes, transferosomes, and nanocochhelates. The hydrophilic and hydrophobic domain/ segment within the molecular geometry of amphiphilic lipids orient and self organize in ordered supramolecular structure when confronted withsolvents [15]. Some commonly used synthetic phospholipids are dioleoyl- phosphatidyl- choline (DOPC), dioleoyl-phosphatidyl-ethanolamine (DOPE), distearoyl-phosphatidyl-choline (DSPC), distearoyl-phosphatidyl-ethanolamine (DSPE) [16].

Among these all phospholipids, phosphatidylcholine classes of phospholipids are very important in the drug delivery technology. The very first and most important advantage of phospholipids based vesicular system is the compatibility of phospholipids with membrane of human either internal membrane as well as skin.

Advantages of phospholipids based carrier system [16] in comparison to other delivery systems:-

1.) These systems show enhanced permeation of drug through skin for transdermal and dermal delivery.

2.) These are platform for the delivery of large and diverse group of drugs (peptides, protein molecules).

3.) Their composition is safe and the components are approved for pharmaceutical and cosmetic use.

4.) Low risk profile- the toxicological profiles of the phospholipids are well documented in the scientific literature.

5.) High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.

6.) The vesicular system is passive, non-invasive and is available for immediate commercialization.

Mechanism of Phytophospholipid Complex Formation: The poor absorption of flavonoid nutrients 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 the standardized extract or polyphenolic constituents (like simple flavonoids) in anon polar solvent. 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 these compounds while the lipid soluble phosphatidyl portion comprising the body and tail which then envelopes the choline bound material. Hence, the phytoconstituents produce a lipid compatible molecular complex with phospholipids, also called as phytophospholipid complex.

Molecules are anchored through chemical bonds to the polar choline head of the phospholipids, as can be demonstrated by specific spectroscopic techniques. Precise chemical analysis indicates the unit phytosomes is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule. The result is a little microsphere or cell is produced [17].

1. **Planterosome Technology:** The poor absorption of flavonoid nutrients 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, flavonoid molecules typically have poor miscibility with oils and entrocytes, the cell that line the small intestine. The planterosomes technology meets this challenge. Certain of the water-phase flavonoid molecules can be converted into lipid-compatible molecular complexes, aptly called planterosomes.

These are better able to transition from the water phase external to the enterocyte, into the lipid phase of its outer cell membrane and from there into the cell, finally reaching the blood. The lipid-phase substance employed to form molecular complexes with phospholipids from soya, mainly phosphotidylcholine (PC). phospholipids are small lipid molecules where glycerol is bonded to two fatty acids, while the third hydroxyl, normally and of the two primary methylenes, bears a phosphate group bound to a biogenic amino or to an amino acid thus making planterosomes different from liposomes. PC is the principal molecular building block for cell membranes and the molecular properties that suit PC for this role also render it close to ideal for its planterosome role.

PC is miscible both in the water phase and in oil/lipid phase, and is excellently absorbed when taken by mouth, and has the potential to act as a chaperon for polyphenolics, shuttling them through biological membranes. Precise chemical analysis indicates the unit planterosome is usually a flavonoid 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 entrocyte's outer cell membrane. Once there, it can cross the entrocyte and reach the circulating blood. The flavonoid and terpenoid constituents of plant extract lend themselves quite well for the direct binding to phosphatidylcholine.

Planterosomes result from the reaction of a stoichiometric amount of the phospholipids (phosphatidylcholine) with the standardized extract or polyphenolic constituents (like simple flavonoid) in a non polar solvent [18]. The formation of lipid molecular complex results in a formation of a little micro sphere or cell is produced and this can be demonstrated by specific spectroscopic techniques [19, 20]. The planterosome technology produce a little cell, whereby the plant extract or its active constituents is protected from destruction by gastric secretions and gut bacteria owing to the gastroproctective property of phosphatidylcholine [21].

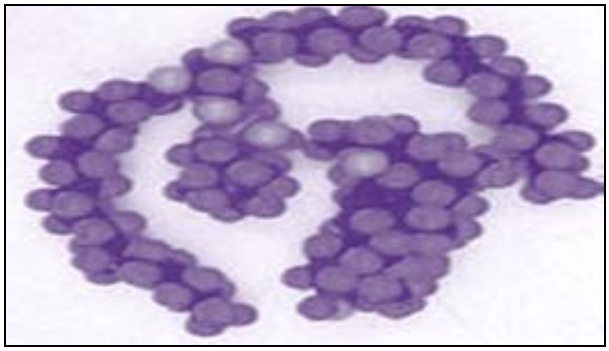


FIG. 2: ORGANIZATION OF THE PLANTEROSOME MOLECULAR COMPLEX

A flavonoid molecule (lower right) is enveloped by a phospholipid molecule

2. **Difference between Planterosomes (Phytosome) and Liposomes:** The fundamental difference between liposomes and planterosomes is that in liposomes the active principle is dissolved in the medium contained in the cavity or in the layers of the membrane, whereas in the planterosome it is an integral part of the membrane, being the molecules anchored through chemical bonds to the polar head of the phospholipids (fig. 1). Liposomes are used primarily in cosmetics to deliver water-soluble substance to the skin.

   A liposome is formed by mixing a water-soluble substance. There may be hundreds or even thousands of phosphatidylcholine and the individual plant components actually from a 1:1 or a 2:1 complex depending on the substance. On the contrary, in a planterosomes, the active principle can somehow be compared to an integral part of the lipid membrane. Furthermore, in liposomes the content of phospholipids is much higher; about five times the one in planterosomes, making this delivery from not suitable for oral clinical realistic dosages for natural compounds. This difference result in planterosomes being much better absorbed than liposomes. Planterosomes are superior to liposomes in skin care products [22, 23].

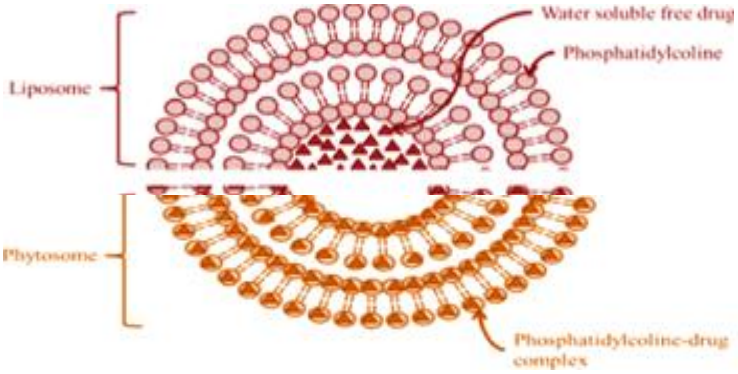


FIGURE 3: DIFFERENCE BETWEEN PLANTEROSOME (PHYTOSOMES) AND LIPOSOME

The molecular organization of planterosomes (lower segment) liposome (upper segment)

Properties of Planterosome:

1. **Physico-Chemical properties:** Planterosomes is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex is obtained by reaction of stoichiometric amounts of phospholipids and the substrate in an appropriate solvent. On the basis of spectroscopic data it has been shown that the main phospholipids-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. When treated with water, herbosome assumes a micellar shape forming liposomal-like structures.

   In liposomes the active principle is dissolved in the internal pocket or it is floating in the layer membrane, while in herbosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane. For example in the case of the catechin distearoyl phosphotidylcholine complex, there is the formation of H-bonds between the phenolic hydroxyl ends of the flavones moiety and the

phosphate ion on the phosphatidylcholine moiety. Phosphatidylcholine can be deduced from the comparison of 1H-NMR and 13C-NMR spectra of the complex with those of the pure precursors. The signals of fatty chain remain almost unchanged. Such evidence inferred that the too long aliphatic chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipids and flavanoid molecules and enables the complex to dissolve in low polarity solvent [24, 25].

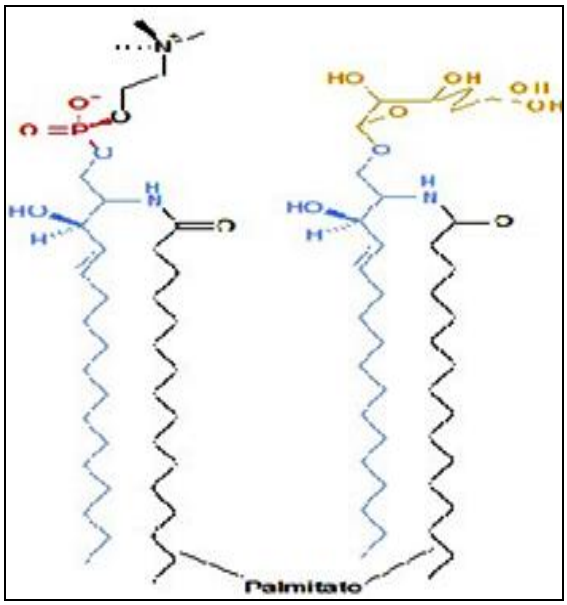


FIGURE 4: STRUCTURE OF PHOSPHATIDYLCHOLINE

2. **Chemical properties:** A planterosomes is a complex between a natural product and natural phospholipids, like soy phospholipids. They are formed due the interaction of hydrogen bonds between the polar head of phospholipids and the polar functional groups of the substrate. They are lipophilic substances with a clear melting point, freely soluble in non polar solvents (in which hydrophilic moiety was not), and moderately soluble in fats. When treated with water, planterosomes assume a liposome like structures In which plant extract linked through hydrogen bonds with polar head of phospholipids [23, 26, 27] and can be easily demonstrated by specific spectroscopic techniques [28, 29].

3. **Biological properties:** Pharmacokinetic and pharmacodynamic studies in experimental animals and in human subjects have been used to demonstrate the biological behavior of planterosomes [17]. The increased bioavailability of the herbosomes over the non complexed botanical derivatives has been evaluated from these studies [23].

Merits of Planterosomes over Conventional Dosage Forms: There is a dramatic enhancement of the bioavailability of botanical extract due to their complexation with phospholipids and improved absorption.

- They permeate the non-lipophilic botanical extract to allow better absorption from the intestinal lumen, which is otherwise not possible [30]. The formulation of planterosomes is safe and the component has all been approved for pharmaceutical and cosmetic use [23, 24].

- They have been used to deliver liver-protecting flavonoid because they can be made easily bioavailable by Planterosomes [31]. In addition to this phosphatidylcholine is also hepatoprotective and so provide a synergistic effect for liver protection. This technology offers cost-effective delivery of phytoconstituents and synergistic benefits. They can also be used for enhanced permeation of drug through skin for transdermal and dermal delivery [32].

- Phosphatidylcholine, an essential part of the membrane used in planterosome technology, act as a carrier and also nourishes the skin. There is no problem with drug entrapment during formulation preparation. Also, the entrapment efficiency is high and more over predetermined, because the drug itself forms vesicle after conjugation with lipid. They offer a better stability profile because chemical bonds are formed between the phosphatidylcholine molecule and phytoconstituents.

- The planterosomal system is passive, non-invasive and can is suitable for immediate commercialization. The dose requirement is reduced due to improved absorption of the main constituent. They can also be given in smaller quantities to achieve the desired result. Relatively simple to manufacture with no

complicated technical investment required for the production of planterosomes.

- Planterosomes are also superior to liposomes in skin care products while the liposomes are an aggregate of many phospholipids molecules that can enclose other phytoactive molecules but without specifically bonding to them. Liposomes are tout delivery vesicles but, for dietary supplement, their promise has been fulfilled.

However, in the case of planterosomes products, numerous studies have proved that they are markedly better absorbed and have substantially greater clinical efficacy. Companies have successfully applied this technology to a number of standardized flavonoid preparations.

Advantages of Planterosomes:

1. It enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability, hence significantly greater therapeutic benefit.

2. As the absorption of active constituents is improved, its dose requirement is also reduced phosphatidylcholine used in preparation of planterosomes, besides acting as a carrier also acts as a hepatoprotective, hence giving the synergistic effect when hepatoprotective substances are employed.

3. Chemical bonds are formed between phosphatidylcholine molecule and phytoconstituent, so the plantersomes show better stability profile. Added nutritional benefit of phospholipids and marked enhancement of bioavailability.

4. Planterosomes process produces a little cell whereby the valuable components of the herbal extract are protected from destruction by digestive secretion and gut bacteria. Assured delivery to the tissues. No compromise of nutrient safety.

5. Dose requirement is reduced due to absorption of chief constituent.

6. Entrapment efficiency is high and more over predetermined because drug itself in conjugation with lipids is forming vesicles so no problem of drug entrapment.

7. Planterosomes show better stability profile because chemical bonds are formed between phosphatidylcholine molecules and phytoconstituents.

8. Phosphatidylcholine used in the planterosomes process besides acting as a carrier also nourishes the skin, because it is essential part of cell membrane.

9. Planterosomes are also superior to liposomes in skin care products. Significantly greater clinical benefit. The particular structure of planterosomes elicits peculiar properties and advantages in cosmetic application.

Preparation methods: Planterosomes are formulated by patented processes in which the standardized extract (having a standardized content of active principles) and/or active ingredients of herbs (like flavoliganans and terpenoids) are bound to the phospholipids like phosphatidylcholine (PC) through a polar end. The planterosomes process produces small cells which protect the valuable components of the herbal extracts are well suited to direct binding to phosphatidylcholine from soy.

PC is also the principle molecular building block of cell membranes and is miscible with both water and oil/lipid mixtures, and is well absorbed orally. Planterosomes are also considered as a phytolipid delivery system. Planterosomes are prepared by reacting 3-2 moles (Preferably with one mole) of a natural or synthetic phospholipids, such as phosphatidylcholine, phosphatidylethanolamine or phytoconstituent is 1:1. The complex thus formed can be isolated by precipitation with an aliphatic hydrocarbon or lyophilization or spray drying [33].

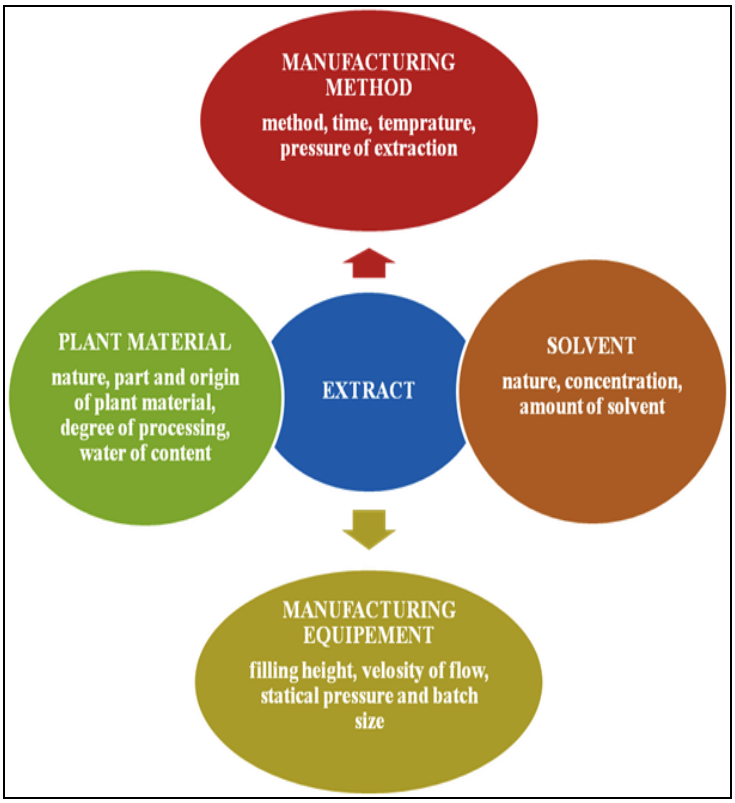
Mareno and lampertico (1991) [34] Jiang *et al* (2001) [35], Maiti *et al* (2006) [36], Maiti *et al* (2006)[37], have described the methods used for herbosome preparation. Jiang *et al.* have optimized the preparation conditions using a uniform design and step regression and have prepared *herba Epimedii* total flavonoid phytosomes (EFP) by means of solvent evaporation and investigated the cumulative dissolution of different ratios of EFP-PVP precipitates by means of dissolution release. The optimized preparation conditions are as follows: solvent-tetrahydrofuran, lecithin to PVP ratio-2.5, temperature-40°C and reaction time-3 hrs. The oil/water apparent partition coefficient of icariin was enhanced more than 4-fold by phospholipids. The cumulative dissolution of *Herba Epimedii* flavonoid of the EFP-PVP precipitate was significantly higher than that of its physical mixture and a *Herba Epimedii* extract tablet.

Yanyu et al prepared a silybin-phospholipids complex using ethanol as a reaction medium. Silybin and phospholipids were resolved into the medium, after the organic solvent was removed under vacuum condition, and a silybin-phospholipids complex was formed.

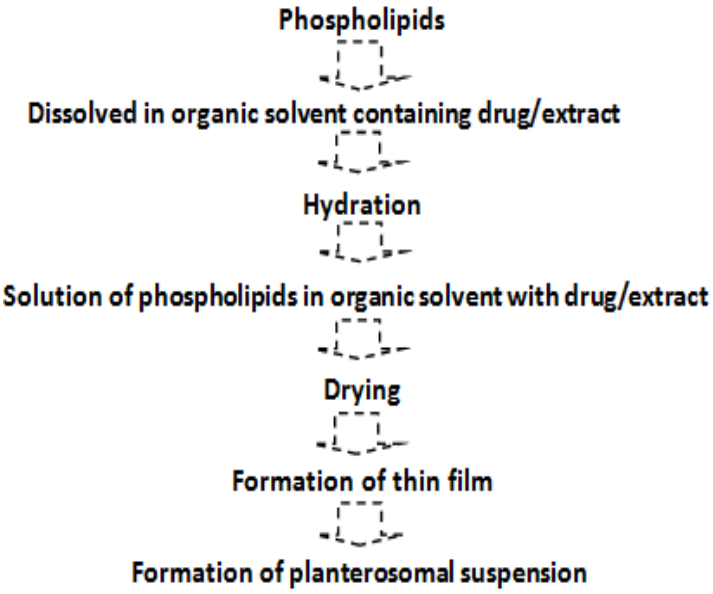
Naringenin-PC complex was prepared by taking naringenin with an equimolar concentration of phosphatidylcholine (PC). The equimolar concentration of PC and naringenin were placed in a 100 ml round bottom flask and refluxed in dichloromethane for 3 h. on concentrating the solution to 5-10 ml, 30 ml of n-hexane was added to get the complex as a precipitate followed by filtration. The precipitate was collected and placed in vacuum desiccators [38].

The required amount of the drug and phospholipids were placed in a100 ml round-bottom flask and dissolved in anhydrous ethanol. After ethanol was evaporated off under vacuum at 40°C, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant silybin-phospholipids complex was transferred into a glass bottle, flushed with nitrogen and stored in the room temperature [39].

Preparation methodology:



Common stages for preparation of Planterosome [40]:

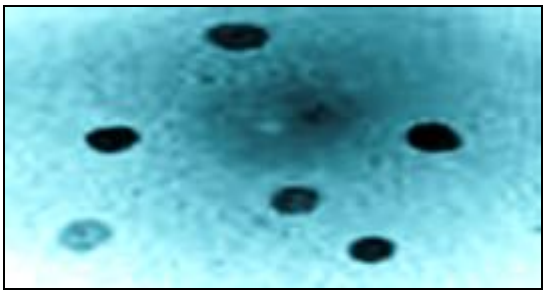


Characterization and Evaluation of Planterosomes: The behavior of planterosomes in both physical and biological systems is governed by factors such as the physical size, membrane permeability, percentage of entrapped solutes, and chemical composition as well as the quantity and purity of the starting material. Planterosomes can be characterized in terms of their

physical attributes i.e. shape, size, distribution, percentage drug captured, entrapped volume, percentage drug released and chemical composition [41]

Visualization: Visualization of planterosomes can be achieved using Transmission Electron Microscopy (TEM) and by Scanning Electron Microscopy (SEM) electron microscopic techniques used to assess liposome shape and size are mainly negative-stain transmission microscopy and scanning electron microscopy. The later technique requires dehydration of the sample prior to examination and is less preferred.

Negative stain electron microscopy visualizes relatively electron transparent liposomes or planterosomes as bright area against a dark background (hence termed as negative stain). Liposome like structure is embedded in this method in a thin film of electron-dence heavy metal (salt) stain. The use of negative stain electron microscopy facilitates estimation of the liposome size range at the lower end of the frequency distribution. Irregular or ellipsoid shape can be treated mathematically to correct for perimeter irregularities thus estimations of original spherical diameter can be calculated [42].



Vesicle 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) [43].

Entrapment efficiency: The entrapment efficiency of a drug by planterosomes can be measured by the ultracentrifugation technique [44].

Transition temperature: The transition temperature of the vesicular lipid systems can be determined by differential scanning calorimeter [45]

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 [46].

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 [47].

Drug content: The amount of drug can be quantified by a modified high performance liquid chromatographic method or by a suitable spectroscopic method [48].

Spectroscopic Evaluations: To confirm the formation of a complex or to study the reciprocal interaction between the phytoconstituent and the phospholipids, the following spectroscopic methods are used [49];

1. **^{1}H-NMR:** The NMR spectra of (+)-catechin and its stiochiometric complex with distearoyl phosphatidylcholine have been studied by Bombardelli *et al.* in polar solvents, there is amarked change of the 1H-NMR signal originating from the atoms involved in the formation of the complex, without any summation of the signal peculiar to the individual molecules. The signals from the protons belonging to the flavonoid are to be broadened that the proton cannot be relieved. In the phospholipids, there is broadening of all the signals while the singlet corresponding to the N-$(CH_3)_3$ of choline undergo an uplift shift. Heating the sample to 60°C results in the appearance of some new broad bands, which correspond mainly to the resonance of the flavonoid moiety.

2. **^{3}C-NMR:** In the 13C-NMR spectrum of (+)-catechin and its stoichiometric complex with distearoyl phosphatidylcholine, particularly when recorded in C6D6 at room temperature, all the flavonoid carbons are clearly invisible. The signals corresponding to the glycerol and choline portion of the lipid (between 60-80ppm) are broadened and some are shifted, while most of the resonance of the fatty acid chain retains their original sharp line shape.

After heating to 60°C, all the signal belonging to the flavonoid moieties reappear, although they are still very broad and partially overlapping.

3. **FTIR:** The formation of the complex can be also be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectroscopy is also a useful tool for the control of the stability of planterosomes when micro-dispersed in water or when incorporated in very simple cosmetic gels. From a practical point of view, the stability can be confirmed by comparing the spectrum of the complex in the solid form (planterosomes) with the spectrum of its micro-dispersion in water after lyophilization, at different times. In the case of simple formulations, it is necessary to subtract the spectrum of the excipients (blank) from the spectrum of the cosmetic form at different times, comparing the remaining spectrum of the complex itself.

4. ***In-vitro* and *in-vivo* Evaluation:** 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 planterosomes. For example, in-vitro antihepatotoxic activity can be assassed by the antioxidant and free radical scavenging activity of the planterosomes.

For assessing antihepatotoxic activity *in-vivo*, the effect of prepared planterosomes on animals against thioacetamide, paracetamolor alcohol-ointment, a commercial product, describe the in vivo safety evaluation methodology [50].

Filburn *et al.,* studied the bioavailability of a silybin phosphatidylcholine complex in dog model to examine the pharmacokinetic parameters of this new complexed form [51].

Pharmaceutical Scope of Planterosomes [52-56]:

1. It enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability, hence significantly greater therapeutic benefit.

2. Appreciable drug entrapment.

3. As the absorption of active constituent(s) is improved, its dose requirement is also reduced.

4. Phosphatidylcholine used in preparation of planterosomes, besides acting as a carrier also acts as a hepatoprotective, hence giving the synergistic effect when hepatoprotective substances are employed.

5. Chemical bonds are formed between phosphatidylcholine molecule and phytoconstituent, so the planterosomes show better stability profile.

6. Application of phytoconstituents in form of planterosome improves their percutaneous absorption and act as functional cosmetics.

Commercial preparations of Planterosomes (phytosomes) and their uses [57-59]:

Trade name	**Phytochemical**	**Indication**
18ß-glycyrrhetinic acid phytosomes	18ß-glycyrrhetinic acid from licorice rhizome	Soothiing
Centella phytosomes	Triterpenes from centella asiatica leaf	Cicatrizing, trophodermic
Crataegus phytosomes	Vitexin-2”-O-rhamnosides from hawthorn flower	Antioxidant
Escinß-sitosterol phytosomes	Escinß-sitosterol from horse chestnut fruit	Anti-oedema
Ginkgoselect phytosomes	Ginkgoflavonglucosides, ginkgolides, bilobalides from ginkgo biloba leaf	Vasokinetic
Ginselect phytosomes	Ginsenosides from panax ginseng rhizome	Skin elasticity improver, adaptogenic
Ginkgo biloba terpenes phytosomes	Ginkgolides and bilobalide from ginkgo biloba leaf	Soothing
Ginkgo biloba dimeric flavonoids phytosomes	Dimeric flavonoids from ginkgo biloba leaf	Lipolytic, vasokinetic

Greenselect phytosomes	Polyphenols from green tea leaf	Prevention of free radical-mediated tissue damages and weight management
Leucoselect phytosomes	Polyphenol from grape seed	Antioxidant, capillarotropic
Meriva	Curcuminoids from turmeric rhizome	
PA_2 phytosomes	Proanthocyanidin A_2 from horse chestnut bark	Anti-wrinkles, UV protectant
Sericosides phytosomes	Sericoside from terminalia sericea bark root	Anti-wrinkles
Siliphos	Silybin from milk thistle seed	Hepatocyte protection
Silymarin phytosomes	Silymarin from milk thistle seed	Antihepatotoxic
Virtiva	Ginkgoflavonglucosides, ginkgolides, bilobalide from ginkgo biloba leaf	Vasokinetic
Visnadex	Visnadin from amni visnaga umbel	Vasokinetic
Mirtoselect phytosomes	Anthocyanosides of bilberry	Potent antioxidant
Sabalselect phytosomes	Saw palmetto berries	Benefit non-cancerous prostate enlargement
LymphaselectTM phytosomes	Melilotus officinalis	For venous disorders, including chonic venous insufficiency of the lower limbs
oleaselectTM phytosomes	Olive oil polyphenol	Anti oxidant, anti inflammatory, anti hyperlipidemic
PolinaceaTM	Echinacea angustifolia	Neutraceutial, immunomodulator

Therapeutic Applications of Planterosomes (Phytosomes) [60, 61, 62]:

Phytosomes	Phytoconstituent complexed with PC	Daily dosage	Indications
Leucoselect phytosomes	Procyanidolic oligomers from grape seed	50-100mg	Systemic antioxidant, specific. Best choice for most people under age of fifty. Also specific for the eyes, lungs, diabetes, varicose veins, and protection against heart disease
Greenselect phytosomes	Epigallocatechin 3-O-gallate from camellia sinensis(green tea)	50-100mg	Systemic antioxidant. Best for protection against cancer and damage to cholesterol
Silybin phytosomes	Silybin from silymarin (milk thistle	120mg	Best choice if the liver or skin needs additional antioxidant protection
Siliphos TM milk thistle phytosomes	Silybin from silymarin	150mg	Good choice for liver or skin support
Hawthorn phytosomes	Flavonoid	100mg	best choice in heart disease
Panax ginseng phytosomes	37.5% ginsenosides from roots of panax ginseng	150mg	As a food product
Ginkgoselect phytosomes	24% ginkgo flavono glycosides	120mg	Best choice for most people over the age of 50. Protect brain and vascular lining

Some Patented Technologies related to Planterosomes (Phytosomes):

Title of patent	Innovation	Patent No.	Reference
Phospholipid complexes of olive fruits or leaves extract having improved bioavailability	Phospholipids complexes of olive fruits or leaves extract or composition containing it having improved bioavailability.	EP/1844785	63
Composition comprising ginkgo biloba derivatives for the treatment of asthmatic and allergic conditions	Composition containing fractions deriving from *Ginkgo biloba*, useful for the treatment of asthmatic and allergic conditions.	EP/1813280	64
Fatty acid monoesters of sorbityl furfural and composition 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.	EP/1690862	65
Cosmetic and dermatologial 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 fibroblast cosmetic or dermatological composition for topical treatment.	EP/1640041	66
Treatment of skin, and wound repair, with thymosin beta	Compositions and methods for treatment of skin utilizing thymosin ß4	US/2007/045541	67

Soluble isoflavone compositions	Isoflavone compositions exhibiting improved solubility, taste, color, and texture characteristics, and methods for making the same.	WO/2004/045541	68
An anti-oxidant prepration based on plant extract for the treatment of circulation and adiposity problems	Prepration based on plant extract which has an anti-oxidant effect and is particularly useful in the treatment of circulation problems such phlebitis, varicose veins, arteriosclerosis, haemorrhoids and high blood pressure	EP1214084	69
Complexes of saponins with phospholipids and pharmaceutical and cosmetic composition contaiong them	Complexes of saponins with natural or synthetic phospholipids have lipophilic and improved bioavailability and are suitable for use as active principle in pharmaceutical, dermatologic, cosmetic compositions	EP0283713	70

Improved Bioavailability, the Recent Research: Most of the planterosomes phytosomal studies are focused to *Silybum marianum* which contain premier liver-protectant flavonoid. The fruit of the milk thistle plant (*S. marianum*, Family- Steraceae) contains flavonoid known for hepatoprotective effect. Silymarin has been shown to have positive effects in treating liver diseases of various kinds, including hepatitis, cirrhosis, fatty infiltration of the liver (chemical and alcohol induced fatty liver) and inflammation of the bile duct. The antioxidant Capacity of silymarin substantially boosts the liver's resistance to toxicity [71]. Silymarin primarily contains three flavonoid of the flavonol subclass (having a fully saturated C-ring).

Silybin is actually a flavonolignan, probably produced within the plant by the combination of a flavonol with a coniferyl alcohol. It is now known that silybin is the most potent of the three [72] Silybin protects the liver by conserving glutathione in the parenchymal cells [71], while PC helps repair and replace cell membrane [73]. Recent research shows improved absorption and bioavailability with phytosomes as compared to the conventional means. Most of the phytosomal or planterosomal studies are focused to silybum marianum (milk thistle) which contains premier liver-protectant flavonoid. The fruits of the milk thistle plant contain flavonoids known for hepatoprotective effects [74, 75].

Francesco & co- associates (2009): studied on a recently developed oral formulation in the form of coated tablets (Monoselect Camellia®) (MonCam) containing highly bioavailable green tea extract (GreenSelect® phytosomes) was tested in obese subjects (n=100) of both gender on a hypocaloric diet. Fifty subjects were assigned to the green tea extract plus hypocaloric diet, while the others 50 subjects followed the hupocaloric diet only. After 90 days of treatment, significant weight loss and decreased body mass index (BMI) were observed in the group taking the herbal extract (14 kg loss in the green tea group compared to a 5 kg loss in the diet-only group); waistline was reduced only in male subjects. Besides the effect on weight and BMI, biochemical parameters (LDL, HDL, and total cholesterol, triglycerides, growth hormone, insulin like growth factor-1, insulin, and cortisol) were improved in both groups. Leptin, nnot tested in the diet-only group, was reduced in patients taking Mon Cam. Taking into consideration the high safety profile of the product and the total absence of adverse effects observed during and after the trial, Mon Cam appears to be a safe and effective tool for weight loss [76].

Mukerjee & co- associates (2008): Hesperetin is a potent phytomolecule abundant in citrus fruits, such as grapefruit and oranges. In spite of several therapeutic benefits viz. antioxidant, lipid-lowering, anti-carcinogenic activities their shorter half life and lower clearance from the body restricts its use. To overcome this limitation, recently Mukerjee et al. developed a novel hesperetin phytosome by complexing hesperitin with hydrogenated phosphatidyl choline. This complex was then evaluated for antioxidant activity in CCl4 intoxicated rats along with pharmacokinetic study revealed that the phytosome had higher relative bioavailability than that of parent molecule at the same dose level [77].

Yanyu & co- associates (2006): prepared the silymarin phytosome and studied its pharmacokinetics in rats. In the study the bioavailabilitymof silybin in rats was increased remarkably after oral administration of

prepared silybin-phospholipids complex due to an impressive improvement of the lipophilic property of silybin-phospholipids complex and improvement of the biological effect of silybin [78].

Maiti & co- associates (2006): developed the phytosomes of curcumin (flavonoid from turmeric, curcuma longa) and naringenin (a flavonoid from grape fruit, vitis vinifera) in two different studies [79, 80].

Maiti & co- associates (2005): developed the quercetin phospholipids phytosomal complex by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecules in rat liver injury induced by carbon tetrachloride [81]

Ravarotto & co- associate (2004): reported silymarin phytosomes show better anti-hepatotoxic activity than silymarin alone and can provide protection against the toxic effects of aflatoxi B1 on performance of broiler chicks [82].

Tedesco & co- associate (2004): reported silymarin phytosome show better anti-hepatotoxic activity than silymarin alone and can provide protection against the toxic effects of aflatoxin B1 on performance of broiler chicks [83]. Busby *et al*., reported that the use of a silymarin phytosome showed a better fetoprotectant activity from ethanol-induced behavioral deficits than uncomplexed silymarin [84].

Grange & co- associate (1999): conducted a series of studies on silymarin phytosome, containing a standardized extract from the seeds of S. marianum, administered orally and found that it could protect the fetus from maternally ingested ethanol [85] grape seed phytosome is composed of oligomeric polyphenols (grape proanthocyanidins or procyanidins from grape seed extract, vitis vinifera) of varying molecular size, complexed with phospholipids. The main properties of procyanidin flavonoid of grape seed are an increase in total antioxidant capacity and stimulation of physiological antioxidant defenses of plasma, protection against atherosclerosis thereby offering marked protectionfor the cardiovascular system and other organs through a network of mechanisms that extends beyond their great antioxidant potency [86].

Moscarella & co- associates (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 functions returned to normal faster in patients taking silybin phytosomes compared to a group of controls (49 treated with commercially available silymarin, 117 untreated or given placebo) [87].

Bombardelli & co- associate (1991): reported silymarin phytosomes, in which silymarin (a standardized mixture of flavanolignans extracted from the fruits of *S. marianum*) was complexed with phospholipids. 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 14. In the human subjects silybin from phytosomes effectively reaches the intended target organ, the liver [88].

Barzaghi & co- associate (1990): conducted a human study designed to assess the absorption of silybin when directly bound to phosphatadylcholine. 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) [89].

Schandalik & co- associate: used nine volunteer patients who had earlier undergone surgical gall bladder removal necessitated by gallstones. They received single oral doses of 120 mg silybin as silybin phytosomes, and bile was accessed for silybin levels. Silybin appeared in the bile after 48 hours accounted for 11 percent of the total dose. In the case of silymarin, approximately 3 percent of the silybin was recovered.

These data demonstrates a four times greater passage through the liver for phytosomal silybin studies have approximately 3 percent of the silybin was recovered. These data demonstrate a four times greater passage through the liver for phytosomal silybin studies have

shown ginkgo phytosome (prepared from the standardized extract of ginkgo biloba leaves) produced better results compared to the conventional standardized extract from plant (GBE, 24 % ginkgo flavones glycoside and 6% terpene lactones).

In a bioavailability study conducted with healthy human volunteers the levels of GBE constituents (flavonoid and terpenes) from the phytosomal form peaked after 3 hours and persisted longer for at least 5 hours after oral administration. It was found that the phytosomal GBE produced 2-4 times greater plasma concentration of terpenes than did the non phytosomal GBE. Their major indications are cerebral insufficiency and peripheral vascular disorder, and it also can ameliorate reduced cerebral circulation. Its improved oral bioavailability and good tolerability makes it the ideal ginkgo product even for long term treatment. In studies with ginkgo phytosomes 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 [90].

Green tea extract generally contains a totally standardized polyphenolic fraction (not less than 66.5%, containing epigallocatechin and its derivatives) obtained from green tea leaves (*Thea sinensis*) and mainly characterized by the presence of epigallocatechin 3-O-gallate, the key compound. These compounds are potent modulators of several biochemical processes linked to the breakdown of homeostasis. Green tea has got several long beneficial activities such as antioxidants anticarcinogenic, antimutagenic, antiatherosclerotic, hypochle-sterolemic, cardioprotective, and antibacterial and anticariogenic effects.

Despite such potential actions green tea polyyphenols have very poor oral bioavailability from conventional extracts. The complexation of green tea polyphenols with phospholipids strongly improves their poor oral bioavailability. A study on absorption of phytosomal preparations was performed in healthy human volunteers along with non complexed green tea extract following oral administration. Over the study period of 6 hours the plasma concentration of total flavonoid was more than doubled when coming from the phytosomal versus the non-phytosomal extract. Antioxidant capacity was measured as TRAP (Total Radical-trapping Antioxidant Parameter). The peak antioxidant effect was a 20% enhancement and it showed that the phytosome formulation had about double the total antioxidant effect [91, 92].

In another study, rabbits were fed with a high cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol and induce atherosclerotic lesions in their feed for the first 6 weeks, than 4weeks of the high-cholesterol diet. These developed significantly less aortic plaque than did the control groups which received conventional standardized grape seed extract in similar regimen. In a randomized human trial, young healthy volunteers received grape seed phytosomes once daily for 5 days. The blood TRAP (Total Radical-trapping Antioxidant Parameter) was measured at several time intervals during 1st day, then also on 5th day. Already by 30 minutes after administration on 1st day, blood TRAP levels were significantly elevated over the control which received conventional standardized grape seed extract [93].

CONCLUSION: Planterosomes are novel formulations which offer improved bioavailability of hydrophilic flavonoids and other similar compounds through the skin or gastrointestinal tract. They have many distinctive advantages over other conventional formulations. As far as the potential of planterosome technology is concerned, it has a great future for use in formulation technology and applications of hydrophilic plant compounds.

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 planterosome showing much better absorption profile following oral administration owing to improved lipid solubility which enables them to cross the biological membrane, resulting enhanced bioavailability i.e. more amount of active principle in the systemic circulation.

This means 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. Hence, the therapeutic action becomes enhanced,

moredetectable and prolonged. Several excellent phytoconstituents have been successfully delivered in this way exhibiting remarkable therapeutic efficacy in animal as well as in human models.

Recently Mukherjee & co associates have regarded planterosomes as a value added drug delivery system. Thorough study of literature reveals that several plant extracts (crude, partially purified or fractionated) are reported to possess different significant pharmacological or health promoting properties. These extracts can be standardized accordingly and may be formulated as planterosomes for systematic investigation for any improved potential to be used rationally. In this way after screening and selection of potential extracts or constituents from plants, planterosomes can be developed for different therapeutic purposes like cardiovascular, anti-inflammatory, immunomodulator, anticancer, antidiabetic etc or for prophylactic and health purposes as nutraceuticals, in due course.

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