INTRODUCTION
Preparations of phytomedicine has been used for health maintenance since ancient times. The phytomedicine’s posses a lot of therapeutic uses. Over the past century phytochemical and phyto-pharmacological sciences established the compositions, biological activities and health promoting benefits of herbal extracts and their derivatives. It was observed that most of the biologically active phytocconstituents such as the flavonoids and terpenoids are of highly polar nature or water soluble molecules. These highly water soluble constituents are poorly absorbed due to their poor lipid solubility, thus creating a hurdle to cross the highly lipid-rich biological membrane, which finally results in poor bioavailability. Many approaches have been developed for improving the bioavailability such as inclusion of solubility and bioavailability enhancers, structural modification and entrapement with lipophilic carriers[1-3]. One such approach is the phytosome technology. The phytosome technology is a novel approach developed by Indena in an attempt to combat the issue of poor bioavailability. The term “phyto” means plant and “some” means cell like. This novel preparation comprises of incorporating a standardized plant extract into phospholipids to produce lipid compatible molecular complexes with enhanced absorption and bioavailability. The phytosome process produces a little cell whereby the valuable plant extracts are protected from degradation by digestive enzymes and gut bacteria. Phospholipids are complex molecules responsible for formation of cell membranes. Phospholipids are lipid molecules in which the glycerol is bonded to two fatty acids and the remaining portion occupied by the phosphate group[6]. The phospholipid mostly employed is phosphatidylcholine derived from soybean (Glycine max). Phytosomes are obtained by reacting phosphatidylcholine with herbal extracts in an aprotic solvent. Phytosomes posses improved pharmacokinetic and pharmacological properties as compared to the conventional preparation. The flavonoid and the terpenoid component’s of the herbal extract are able to
directly bind to the phosphatidylcholine moiety hence they are widely prepared. The phytosome process has been widely applied to herbal extract such as milk thistle (Silybum marianum), green tea (Thea sinensis)\(^6\).

**A NOVEL APPROACH: PHYTOSOME**

Phytosomes results from reaction of stoichiometric amount of phospholipid mostly phosphatidylcholine with a standardized herbal extract in an aprotic solvent. Phosphatidylcholine is a bifunctional compound, the phosphatidyl moiety being lipophilic in nature which is the head of the bifunctional compound and the choline moiety which is the tail of the bifunctional compound being hydrophilic in nature. The choline moiety of the phosphatidylcholine bounds to the hydrophilic phytoconstituents, whereas the lipid soluble phosphatidyl portion then envelopes the choline bound complex. As a result a phyto-phospholipid complex is formed with a better lipid solubility. The polar phytoconstituents binds to the choline head by means of a chemical bond. The term "phyto" means plant while "some" means cell like.\(^8\)

The phytosome technology produces a little micro sphere or little cell, which protects the plant extract or its active constituent from destruction by gastric secretion and gut bacteria due to the gastroprotective property of phosphatidylcholine.\(^3\) Fig. 1

**PREPARATION OF PHYTOSOME**

Phytosomes are prepared by reacting 3-2 moles or preferably 1 mole of phosphatidylcholine with 1 mole of active phytoconstituents mostly the flavonoids and the terpenoids in an aprotic solvent such as dioxane or acetone from which complex can be isolated by precipitation with non solvent such as aliphatic hydrocarbons or by lyophilization or by spray drying\(^24\). In the phyto-phospholipid complex formation the ratio between these two components is in the range of 0.5-2 moles. The most preferable ratio of phospholipid to phytoconstituents is 1:1\(^16,17\). The phospholipids mostly selected for phytosome preparations are selected from group consisting of soy lecithin (Glycine max); phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, in which acyl group may be same or different and mostly derived from palmitic, stearic, oleic, linoleic acid. The phospholipid mostly selected is phosphatidylcholine\(^25,26\). Spectroscopic techniques reveal’s that the molecules of the phytoconstituents are bonded to phospholipid moiety by means of a chemical bond\(^29-30\). The common stages for the preparation of phytosomes are mentioned in fig 2.

**PROPERTIES OF PHYTOSOME**

Phytosomes are complex between a natural phytoconstituents and natural phospholipids, like soy phospholipids mostly phosphatidylcholine. These complex results from the reaction of stoichiometric amounts of phospholipids with the phytoconstituents in an aprotic solvent\(^13-16\).

1. Phytosomes can accommodate the active principle that is anchored to the polar head of the phospholipids, which finally becomes an integral part of the membrane. The molecules are
2. Phytosomes are advanced form of herbal drugs which are better absorbed, utilized and which finally leads to better results than conventional dosage form. The increased bioavailability has been demonstrated by the pharmacokinetic studies as well as by pharmacokinetic tests in experimental animals and human subjects.
3. Phytosomes are lipophilic substances with definite melting point, freely soluble in non-polar solvents, and moderately soluble in fats.
4. Phytosomes when treated with water assume a micellar shape, forming structure that resemble liposomes exhibiting fundamental difference.

**CHARACTERIZATION OF PHYTOSOME**

There are various factors such as the physical size, membrane permeability, percentage of entrapped solutes, chemical composition of the preparing materials which play a vital role in determining the behavior of phytosomes in physical and biological system. The following are the characterization techniques used for phytosomes in characterizing its physical attributes

1. Transition temperature: The transition temperature of vesicular lipid system can be determined by differential scanning calorimetry\(^25,26\).
2. Entrapment efficiency: The entrapment efficiency of a phytosomal formulation can be determined by subjecting the formulation to ultracentrifugation technique\(^24\).
3. Vesicle size and Zeta potential: The particle size and zeta potential of phytosomes can be determined by dynamic light scattering which uses a computerized inspection system and photon correlation spectroscopy\(^29-30\).
4. Surface tension activity measurement: The surface tension activity of drug in aqueous solution can be measured by ring method Du Nouy ring tensiometer\(^27\).
5. Spectroscopic evaluation: The spectroscopic evaluations are widely employed in order to confirm the formation of complex between phytoconstituents and the phospholipid moiety as well as to study the corresponding interaction between the two. The widely employed methods are

5.1 $^1$H NMR
The NMR spectra are employed for estimating the complex formation between the active phytoconstituents and the phosphatidylcholine molecule. The NMR spectra of phytosome complex had been studied by Bombardelli. In nonpolar solvents there is a marked change in $^1$H NMR signal originating from atoms involved in the formation of complex, without any summation of the signal peculiar to individual molecules. The signals from protons belonging to the phytoconstituents are broadened. In phospholipids there is broadening of signals while the singlet corresponding to the N-(CH$_3$)$_3$ of choline undergoes an upfield shift$^{[29-30]}$.

5.2 $^{13}$C NMR
In the $^{13}$C NMR of the phytoconstituents and the stoichiometric complex with the phosphatidylcholine when recorded in C$_6$D$_6$at room temperature all the phytoconstituents carbons where invisible. The signals corresponding to the glycerol and choline portion are broadened and some are shifted, while most of the resonance of the fatty acids chains retain their original sharp line shape$^{[29-30]}$.

5.3 FTIR
The spectroscopic evaluation of the formed complex can be confirmed by FTIR simply by comparing the spectrum of the complex and the individual components and that of the mechanical mixtures. FTIR can also be considered as a valuable tool in confirming the stability of the phytosomal complex. The stability can be confirmed by comparing the spectrum of the complex in solid form with that of the spectrum of micro-dispersion in water after lyophilization at different times$^{[29-30]}$.

**PHYTOSOMES AND LIPOSOMES : A COMPARISON**

Liposomes are also prepared by mixing suitable water soluble phytoconstituents in phosphatidylcholine in a definite ratio under suitable conditions. Here no chemical bond is formed, the phosphatidylcholine moiety just anchors the water soluble phytoconstituents as a result of which there may be hundreds or even thousands of phosphatidylcholine molecules surrounding the drug molecule. In case of phytosomes the phosphatidylcholine and the plant constituents form a complex in the ratio 1:1 or 2:1 and the process of phytosome formation involves chemical bond formation whereas the liposomes are completely devoid of the chemical bond formation between the phosphatidylcholine molecule and the phytoconstituents. Due to the lesser composition of the phospholipid content in case of phytosomes the phytosomes are more bioavailable and are absorbed to a better extent than the liposomes$^{[16-18]}$. Fig. 3

**ADVANTAGES OF PHYTOSOMES**
Phytosomes furnishes with the following advantages$^{[13-14]}$:

1. Phytosomes produces a little cell where the valuable components of herbal extracts are protected from destruction by digestive secretions and gut bacteria.
2. It assures proper delivery of drug to the respective tissues.
3. The nutrient safety of the herbal extracts need not be compromised by conveying the herbal drug as means of phytosomes.
4. Dose requirement has been reduced due to the maximum absorption of chief constituents.
5. Marked enhancement in the bioavailability of drug occurs.
6. Entrapment efficiency is high and more over predetermined because drug itself is in conjugation with lipids in forming vesicles.
7. There is no problem in drug entrapment while formulating phytosomes.
8. Phytosomes shows better stability profile due to the formation of chemical bonds between phosphatidylcholine molecules and the phytoconstituents.
9. Phosphatidylcholine used in formulating phytosome process besides acting as a carrier also nourishes the skin as it is an essential part of a cell membrane.
10. Phytosomes are also superior to liposomes in skin care products.
11. Phytosomes proves to be of significantly greater clinical benefit.
12. Phosphatidylcholine used in preparation of phytosomes, besides acting as a carrier also acts as a hepatoprotective as a result it imparts a synergistic effect when hepatoprotective substances are employed.

**APPLICATION OF PHYTOSOME**

Silymarin Phytosome
Yanyu et al. prepared the silymarin phytosome and studied the pharmacokinetics in rats. In the study the bioavailability of silybin in rats was increased remarkably after oral administration of prepared silybin-phospholipid complex due to the impressive improvement of
the lipophilic property of silybin-phospholipid complex and which led to the improved biological effect of silybin[4].

Tedesco et al. reported that silymarin phytosome exhibits better anti-hepatotoxic activity than silymarin alone and can play a vital role in protection against the toxic effects of aflatoxin B1 on performance of broiler chicks[21].

Mascarella et al. investigated that in one study of 232 patients with chronic hepatitis 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 control[9].

Bombardelli et al. reported silymarin phytosome in which silymarin was complexed with phospholipids. Phytosomes showed higher specific activity and a longer lasting action than single constituents with respect to the reduction of oedema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging activity[17].

Barzaghi et al. conducted a human study designed to assess the absorption of silybin when directly bounded to phosphatidylcholine. Plasma silybin levels were determined after administration of single oral dose 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 as compared to the absorption of silybin from regular milk thistle[23].

Grange et al. conducted a series of studies on silymarin phytosome, which contained a standardized extract from the seeds of S.marianum, administered orally and found out that it could protect the fetus from maternally ingested ethanol[22].

Phytosome of green tea

Green tea leaves (Theasinnensis) is characterized by prescence of a polyphenolic compound epigallocatechin 3-O-gallate as the key component. These compounds are potent modulators of several biochemical process linked to the breakdown of homeostasis in major chronic-degenerative diseases such as cancer and atherosclerosis. Green tea also furnishes us with a number of beneficial activities such as antioxidant, anticarcinogenic, antimutagenic, hypocholesterolemic, cardioprotective effects. Inspite of such beneficial activities furnished by polyphenols from green tea extract the polyphenols suffer from the problem of poor bioavailability. The complexation of polyphenols derived from green tea with phospholipids strongly improves the oral bioavailability[5]. A study on absorption of phytosomal preparation 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 flavonoids was more than doubled when comparison was done between the phytosomal and the non-phytosomal preparation was done. 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[9].

Quercetin-phospholipid phytosomal complex

Mail et al. developed the quercetin-phospholipid phytosomal complex by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy as compared to the non-phytosomal conventional preparation in rat liver injury induced by carbon tetrachloride[21].

Phytosomes of grape seed

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 flavonoids of grape seed are an increase in total antioxidant capacity and stimulation of physiological defenses of plasma, protection against ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis thereby offering marked protection against the cardiovascular system and other organs through a network of mechanism that extend beyond their antioxidant effect.

In another study, rabbits were fed with a high cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol level and to induce atherosclerotic lesions in their aortas and carotid arteries. One group of rabbit received grape seed phytosome in their feed for the first 6 weeks, then 4 weeks of high cholesterol diet. These developed significantly less aortic plaque than did the control group which received conventional standardized grape seed extract in similar regimen. In randomized human trial, young healthy volunteers received grape seed phytosome 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[31].

Phytosomes of curcumin
Matti et al. developed the phytosomes of curcumin (flavonoid from turmeric, Curcuma longa linn) and naringenin (flavonoid from grape, Vitis vinifera) in two different studies. The antioxidant activity of complex was significantly higher than pure curcumin in all dose levels tested. In the other study the developed phytosome of naringenin produced better antioxidant activity than the free compound with a prolonged duration of action, which may be due to decrease in the rapid elimination of the molecule from the body[47-48].

Phytosomes of Gingko biloba leaves
Studies have shown that ginkgo phytosome (prepared from 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 level of GBE constituents (flavonoids 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 a 2-4 times greater plasma concentration of terpenes than did the non-phytosomal GBE. Its major indication are cerebral insufficiency and peripheral vascular disorders and it can also ameliorate reduced cerebral circulations. Its improved oral bioavailability and good tolerability makes it the ideal ginkgo product even for long term treatment. Studies with ginkgo phytosomes in patients with peripheral vascular disorders have shown to produce 30-60% greater improvement compared to regular standardized GBE[38]. Studies were also conducted on ginkgo phytosome which yielded better result as compared to the conventional form. For conducting the aforesaid studies ginkgo phytosome was administered for 5 days in guinea pigs, in whom the bronchokonstriction was induced by three different agonists (histamine, PAF and Acetylcholine). The bronchospastic inhibition was measured at the maximum peak, expressed as variations versus the basal values. The result indicated that ginkgo phytosome can not only counteract direct bronchoconstriction but also it posses the tendency to reduce the TXA2 mediated bronchoconstriction of histamine and PAF as compared to the conventional forms, thus indicating the improved efficacy of ginkgo phytosome in combating the allergen induced bronchospasm. Studies have also proved the improved efficacy of ginkgo phytosome over the conventional standardized extract in protecting rat isolated hearts against ischemia. The above mentioned results clearly gives an indication about the upper hand that phytosome posses over the conventional preparations, thus proving it’s utility for herbal phytoconstituents[5-6].

PATENTED TECHNOLOGIES RELATED TO PHYTOSOME
A number of innovative processes has been carried out in the field of phytosomes. The academic scientists are conducting a number of formulation research studies on phytosome, the studies are also conducted by industrial laboratories. These studies encompasses the current areas of research and the recent innovations that can be made possible in phytosomes. Some of the patented technologies of phytosome and other related technologies along with their applications and innovations are listed in table. 1

COMMERCIALLY MARKETED FORMULATION
Phytosome are the advanced form of herbal extract which are better absorbed as compared to the conventional standardized herbal extract. These are the patented technologies developed by INDENA SPA Milan, Italy. A number of herbal standardized extract especially the polyphenolic and the terpenoids fraction are widely formulated as phytosome. Some of the marketed formulations are listed in the table. 2

CONCLUSION
A wide number of phytoconstituents are present in herbal drugs especially the flavonoidal and the terpenoidal fraction furnishes with a number of application. The poor absorption and the poor bioavailability associated with the polar phytoconstituents limits its use. These hindrances can be tackled by formulating an appropriate drug delivery system. Phospholipid based drug delivery system have been found promising for better and effective delivery of drug and can enhance the rate and extent of drug absorption across the lipoidal biomembrane. Phytosome are one of the phospholipid based drug delivery system with a better absorption and stability profile as compared to other phospholipid based drug delivery system. Phytosome can play a vital role in efficient delivery of
phytoconstituents such as the flavones and the xanthones. Apart from the aforesaid use phytosome also has a wide scope in cosmetics as well. Many areas of phytosome will be revealed in the future as part of their pharmaceutical use.

**NOVEL APPROACHES FOR DELIVERY OF HERBAL CONSTITUENTS**

**Liposomes**
Liposomes are artificial microscopic vesicles consisting of an aqueous core enclosed in one or more phospholipid layers, used to convey vaccines, drugs, enzymes or other substances to target cells or organs\(^{[46]}\).

**Nanoparticles**
Nanoparticles are particles of less than 100nm in diameter that exhibit new or enhanced size-dependent properties compared with larger particles of same material\(^{[46]}\).

**Microemulsion**
A thermodynamically stable dispersion of two immiscible liquids, stabilized by surfactants. A microemulsion is an emulsion whose particles are less than 1 micron in size\(^{[46]}\).

**Phytosome**
It is a newly introduced patented technology developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes they are also known as herbosome\(^{[46]}\).

**Transfersomes**
A transfersomes carrier is an artificially designed to be like cell vesicle or a cell engaged in exocytosis and thus suitable for controlled and potentially targeted drug delivery. Transfersome consist of phosphatidylcholine and cholate and are ultra deformable vesicles with enhanced skin penetrating properties\(^{[46]}\).

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Fig. 1: Organisation of phytosome complex
Phospholipid
Dissolve in solution containing organic solvent
Solution of phospholipid in solution containing organic solvent+extract
Drying
Thin Film Formation
Hydration
Formation of Phytosomal complex
Isolation by precipitation (by lyophilization or spray drying)

Fig. 2: Stages of Phytosome preparation[46]

**Liposome**

**Phytosome**
- Water soluble free drug
- Phosphatidylcholine
- Phosphatidylcholine drug complex

Fig. 3: Difference between phytosome and liposome
The molecular organization of liposome upper segment
The molecular organization of phytosome lower segment.
<table>
<thead>
<tr>
<th>Title of patent</th>
<th>Innovation</th>
<th>Patent no.</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Phospholipids complexes of olive fruits or leaves extracts having improved bioavailability</td>
<td>Compositions containing fractions derived from Ginkgo biloba useful for treating asthma</td>
<td>EP/1813280</td>
<td>33</td>
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<tr>
<td>Compositions comprising Ginkgo biloba derivatives</td>
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<tr>
<td>Fatty acids monoesters of sorbityl furfural and compositions for cosmetic and dermatological use</td>
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<tr>
<td>Treatment of skin and wound repair with thymosin β4</td>
<td>Complexation of thymosin β4 along phospholipids for treatment of skin disorder</td>
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<tr>
<td>Soluble isoflavone compositions</td>
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<td>An antioxidant preparation based on plant extracts for the treatment of circulation and adiposity problems</td>
<td>Preparations based on plant extracts which has an antioxidant effect and is particularly useful in treatment of circulation problems such as phlebitis, varicose veins, arteriosclerosis, high blood pressure and haemorrhoids</td>
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<tr>
<td>Complexes of saponins</td>
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<tr>
<td>Complexes of saponins with natural and synthetic phospholipids possess pharmaceutical and high lipophilia and improved cosmetic compositions bioavailability and are suitable for containing them use as active principle in pharmaceutical, dermatologic and cosmetic composition</td>
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Fig. 4: NDDS for delivery of herbal constituents

<table>
<thead>
<tr>
<th>Table 2: Commercially Marketed Formulations</th>
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<tr>
<td>Centella phytosome</td>
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<tr>
<td>Crataegus phytosome</td>
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<tr>
<td>Escin β-sitosterol phytosome</td>
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<tr>
<td>Gingkoselect phytosome</td>
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<tr>
<td>Ginselect phytosome</td>
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<tr>
<td>Ginkgo biloba terpenes phytosome</td>
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<tr>
<td>Ginkgo biloba dimeric phytosome</td>
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<td>Greenselect phytosome</td>
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<tr>
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<td>PA₂ phytosome</td>
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<td>Virtiva</td>
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<td>Visnadex</td>
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60th Indian Pharmaceutical Congress; New Delhi, 2008: 287.