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ABSTRACT
Phytosomes are formulated by patented technology by indena to developed process in which the standardized extract (having a standardize content of active principle and/or active ingredient of herbs like flavor - lignans and terpenoids) are bound to the phospholipids like phosphatidycholine (PC) through a polar end. The term “phyto” means plant while “some” means cell-like. Phytosomes formulation increases the absorption of ingredient, improving their systemic bioavailability. Phytosomes are prepared through the attachment of individual ingredient of herbal extract to phosphotidylycholine resulting in a formulation having higher solubility and hence better absorption leading to promoted pharmacokinetic and pharmacodynamic properties compared to conventional herbal extract. Phytosome technology has been used effectively used to the bioavailability of various herbal extracts including, green tea grape seed, ginseng etc and can be developed for various therapeutic uses or dietary supplement.

KEYWORDS: Phytosomes, Antidiabetic, Phytochemical Screening, Herbal Medicine.

INTRODUCTION
Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both. Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion,
Two major types of diabetes have been defined: Insulin-dependent diabetes mellitus (type 1) and non-insulin-dependent diabetes mellitus (type 2).\(^1\)

Type 1 diabetes accounts for 5% to 10% of diabetes, usually occurs in children’s or young adults, and was commonly termed insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes.\(^1,2\) This disease is caused by autoimmune destruction of the pancreatic β cells that secrete insulin.\(^1,2\) The process involves a smoldering destructive process that can persist for several years and ultimately leads to failure of insulin secretion. This autoimmune process is due to genetic and environmental factors, and many genes contribute to the pathogenesis. During the preclinical phase, a variety of autoimmune antibodies directed against β-cell antigens serve as markers for the prediabetic state, allowing for early detection and possible prevention strategies. Patients with type 1 diabetes require insulin therapy for survival, but blood glucose is still difficult to control, and most patients ultimately develop devastating complications of this disease.

Type 2 diabetes accounts for 90% to 95% of all patients with diabetes and is increasing in prevalence, especially in minority populations. Type 2 diabetes is a heterogeneous, polygenic disorder, and the responsible genes have been identified in selected subtypes of this disease. Multiple diabetes genes exist, and more than 1 gene is likely to be involved in an individual patient. Some of the known environmental factors are obesity, a sedentary lifestyle, and aging. Obesity probably is the major environmental factor contributing to the increasing incidence of type 2 diabetes, and some of the hormonal, genetic, and environmental factors that predispose to obesity have been identified.

1.1: Pathophysiology of Type I and Type II diabetes
1. Signs and symptoms

1.1: Symptoms of Type 1 Diabetes
1. Frequent urination
2. Unusual thirst
3. Extreme hunger
4. Unusual weight loss
5. Extreme fatigue and Irritability

There is a reason why diabetes is termed the silent killer. It is important to bear in mind that these symptoms may be mistaken for an ailment in themselves or for some other disease. The best method to diagnose this condition is to have a blood test taken. And if you have already noticed this symptom, you should see a doctor at the earliest.

1.2 Symptoms of Type 2 Diabetes
1. Excessive Urination and Thirst
2. Increased Hunger
3. Unexplained Weight Gain
4. Irritability and Fatigue
5. Blurred Vision
6. Warning Signs of Diabetes
   a. Decelerated Healing
   b. Skin and Yeast Infections plus Frequent Gum and Bladder Infections

Phytosomes

Phytosome is generally prepared by reacting one or two moles of polyphenolic phytoconstituents and phospholipid. It may be either in the ratio of 1:1 and 1:2. By using phytosomes, one can also achieve enhanced rate and extent of the passage of lipophilic herbal constituents across lipid membrane that explains its character as a carrier as well as acid labile herbal drugs could also be protected in gastrointestinal tract. In general, phytosomes constitute complexes between a herbal active constituent and natural phospholipid. There are number of products which are available in the market that contains phytosomal drug delivery system such as Ginkgo biloba, and Camellia sinensis.

Phytosome is a type of nano-delivery system which consists of molecular-level complexation between phytochemicals and phospholipids. Phytosomes are markedly smaller than
liposomes and have been suggested as a promising strategy for improving the bioavailability of phytoconstituents. Soya phosphatidylcholine (SPC) is a purified phospholipid widely used for the preparation of phytosomes. Also, egg phospholipid (EPL) contains various phospholipid species, such as sphingomyelin, phosphatidylethanolamine, and lysophosphatidylcholine, in addition to phosphatidylcholine. The profile, position, and saturation level of fatty acids esterified in the glycerol backbone also showed significant differences between SPC and EPL. These differences in the phospholipid matrix may influence the structure, bioactivity and performance of phytosomes.

![Phytosomes](image)

**Figure 1: Phytosomes.**

**Preparation and methods of phytosomes**

1. **Antisolvent precipitation technique**

The specific amount of lawsone and soya lecithin were taken into a 100 ml round bottom flask and refluxed with 20 ml of dichloromethane at a temperature not exceeding 60°C for 2 h. The mixture is concentrated to 5-10 ml. Hexane (20 ml) was added carefully with continuous stirring to get the precipitate which was filtered and collected and stored in vacuum desiccators overnight. The dried precipitate is crushed in mortar and sieved through #100 meshes. Powdered complex was placed in amber colored glass bottle and stored at room temperature.\(^{[13-14]}\)

2. **Rotary evaporation technique**

The specific amount of lawsone and soya lecithin were dissolved in 30 ml of tetrahydrofuran in a rotary round bottom flask followed by stirring for 3 hours at a temperature not exceeding 40°C. Thin film of the sample was obtained to which n-hexane was added and continuously
stirred using a magnetic stirrer. The precipitate obtained was collected, placed in amber colored glass bottle and stored at room temperature.\textsuperscript{[13]}

3. Solvent evaporation method

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

Selection of herbal extract

Herbal extracts posses various properties such as photo-protection, hepato-protection, anti-aging, moisturizing, and antioxidant, astringent, anti-irritant, and antimicrobial. Because of such properties they produces healing, softening, rejuvenating, and sunscreen effect on skin and improve pharmacological and pharmacokinetic profile in the body. After detailed literature survey of herbs and correlation of activity of herbal compounds based on chemical classes such as flavonoids, monoterpenes, polyphenols, indols and organosulfides, one can select herbal extracts on the basis of their nature, availability, estimation method, stability and utility of developed formulation as well as reported previous research. 1.8.3 Nature of phytoconstituents Solubility is important criterion for the development of novel formulations. According to the nature of the phytoconstituents, that is hydrophilic or lipophilic, best suitable formulation can be selected.

Selection of dosage form for delivery of phytosomes Suitable type of formulation/dosage form for delivery of phytosomes can be selected based on its potential for improving the effectiveness and efficiency of bioactive compound. The application of dosage form should improve its efficacy regarding continuous action of herbs on systemic effect of human body. The inherent properties of herbal drug such as hydrophilic or hydrophobic, surface characteristics of system such as permeability and charges, degree of biodegradability, and tonicity; release profile and size of the product required of the final formulation need to be taken in to consideration. Phytosome can be formulated for both oral as well as topical use.

Following are few suggested dosage forms for phytosome delivery.
Selection of dosage form

- **Soft gelatin capsules**: The phytosome can be dispersed in oily vehicles (vegetable or semi-synthetic oil) to obtain suspension to be filled in soft gelatin capsules.[20]

- **Hard gelatin capsules**: Phytosomes can be filled in to hard gelatin capsules. A direct volumetric filling process without precompression (precompression might affect the disintegration time) can be applied. The low density of phytosome complex seems to limit the maximum amount of powder (usually not more than 300 mg for size capsule) that can be filled into capsule. With the use of piston pump capsule filling process we can increase amount of powder to be filled in capsule.[21]

- **Tablets**: Due to limited flow ability, stickiness and low apparent density of phytosome complex, a direct compression process can be applied only for lower unitary doses. The phytosome complex should be diluted with 60-70% of excipients to obtain tablets with appropriate characteristics. Wet granulation should be avoided due to the negative effect of water and heat on stability of the phyto-phospholipid complex.[22]

- **Topical dosage form**: Firstly prepare emulsion at low temperature (not higher than 40°C) after that incorporates the phytosome complex into it. The phyto-phospholipid complexes are dispersible in the main lipid solvents employed in topical formulation.

**Characterization and Evaluation of Phytosome**

Phytosomes are characterized for physical attributes, i.e. shape, size, its distribution, percentage drug capture, entrapped volume, percentage drug release, and chemical composition. Hence, behavior of Phytosomes, in both physical and biological systems is governed by factors such as physical size, membrane permeability; percent entrapped solutes, chemical composition, quantity and purit of the starting material.

1. **Evaluation of techniques**

- **Visualization**: Visualization of phytosomes can be achieved using transmission electron microscopy.

- **Entrapment efficiency**: The entrapment efficient of a drug by phytosome can be measured by the ultracentrifugation technique.
Transition temperature: The transition temperature of the vesicular lipid systems can be determined by differential scanning colorimeter.

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.

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 Dynamic Light Scattering (DLS) and structural changes are monitored by Transmission Electron Microscopy (TEM).

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

Vesicle size and Zeta potential: The particle size and zeta potential can be determined by DLS using a computerized inspection system and photon correlation spectroscopy.

Some product of phytosomes available in market

<table>
<thead>
<tr>
<th>Product</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>SILIPHOS™</td>
<td>120 mg</td>
</tr>
<tr>
<td>Milk Thistle Phytosomes</td>
<td>150 mg</td>
</tr>
<tr>
<td>Ginkgo Biloba Phytosomes</td>
<td>120 mg</td>
</tr>
<tr>
<td>Grape Seed Phytosomes</td>
<td>50-100 mg</td>
</tr>
<tr>
<td>Green Tea Phytosomes</td>
<td>50-100 mg</td>
</tr>
</tbody>
</table>

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
   a. Mayer’s Test: Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
   b. Wagner’s Test: Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
   c. Dragendorff’s Test: Filtrates were treated with Dragendorff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
   d. Hager’s Test: Filtrates were treated with Hager’s reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.
2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. Molisch’s Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b. Benedict’s Test: Filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars. Internationale Pharmaceutica Scientia Jan-Mar 2011 Vol 1 Issue 1 103 Prashant Tiwari, et al: Phytochemical screening and Extraction: A Review

c. Fehling’s Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling’s A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a. Modified Borntrager’s Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal’s Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

a. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.
6. Detection of phytosterols
   a. Salkowski’s Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
   b. Libermann Burchard’s test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids
   a. Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
   b. Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and aminoacids
    a. Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
    b. Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

11. Detection of diterpenes
    Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

CONCLUSION
Phytosomes are advanced and novel form of botanicals herbs and phytoconstituents that are better absorbed both orally, topically and transdermally. Phytosomes are basically better absorb comparatively conventional herbal dosage forms and phytosomes are improve
bioavailability of herbal extract. The methods of phytosomes are non-conventional and reproducible phytosomes are to be reported in the future in the prospect of pharmaceutical applications.

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