INTRODUCTION: Multifactorial metabolic diseases, for example, diabetes build up several complications such as immunodeficiency, hyperlipidemia, and hepatic toxicity, etc. Fact from ethnopharmacological studies have shown that Citrullus colocynthis is traditionally used as antibacterial, antifungal, antiviral, hepatoprotective, anti-inflammatory, anti-diabetic, antioxidant and anticancer agent. The proposed theory is phytosomes of crude drug extract of Citrullus colocynthis will be more effective and safe as an anti-diabetic agent. Marketed herbal drugs consist of irrational combinations that make their evaluation for quality control more difficult.

Phytoconstituents, in spite of having exceptional bioactivity in-vitro exhibit less or no in-vivo actions because of their poor lipid solubility that results in high dose therapeutic regimen; phospholipids encapsulation can conquer this difficulty. That is why, the present study was planned to develop phospholipids encapsulated herbal anti-diabetic formulation in the form of phytosomes. The remedial uses of phytoconstituents are very well liked for wellbeing and protection from diseases by various means.

The majority of the bioactive constituents of plants are water-soluble or polar molecules (e.g., tannins, phenolics, flavonoids, and glycosides). Though, water-soluble phytoconstituents are restricted in their effectiveness since they are poorly immersed due to huge molecular size along with poor lipid solubility when applied topically or when taken orally. Flavonoids are constructively efficient for anti-inflammatory, anti-allergic, antiviral, antioxidant and anticancer, etc.
The physicochemical properties and chemical structure of flavonoids validate their rate and extent of absorption, and their biological activities rely on their bioavailability. Very less information exists about the bioavailability of flavonoids in literature. The phytosome technique has appeared as the leading method for improving the bioavailability of phytopharmaceuticals having poor proficiency of solubilizing and passing the biological membranes. Phytosome is a patented technology of Indena where phospholipids are complexed with plant-polyphenolics to improve their bioavailability, because phospholipids are also known as lipid molecules where glycerol is bonded to two fatty acids, such as, phosphatidylcholine, are well known lipophilic moieties that readily can form complex with polyphenolic compounds. Phosphatidylcholine is a chief structural component of every biological membrane.

The aim of the present study is to develop an extract of CC loaded phospholipid complex that can have the perspective to enhance the bioavailability of the herbal drug. The main objective of the study is to develop the phytosome of an extract of CC and increase the solubility and bioavailability of drug by using phytosome. The composite thus prepared was evaluated physico-chemically for crystallinity (X-RD), chemical interaction (FT-IR), surface morphology (SEM), solubility, and dissolution rate study. It is assumed that the developed complex may be suitable to decrease the dose and frequency, hence reduce the toxic or side effect of herbal drug.

MATERIALS AND METHODS:
Materials: The fruit of C. colocynthis had been collected from the region of Punjab and Rajasthan. Authentication no. of C. colocynthis is NISCAIR/RHMD/Consult/2017/3097-46. Then, the fruit was shade dried and powdered.

Methods:
1. Extraction of Drug: 100 gm of each shade-dried powder was macerated discretely in 300 ml dichloromethane (DCM) with discontinuous shaking for three days, then filtered. The remains were further extracted for two times by using the same solvent, and all the three filtrates were collected together. The resultant was air dried in shade and further extracted with ethyl acetate and followed by ethanol similar to the procedure as carried out for the DCM extraction. At the end, from each filtrate, the solvent was evaporated using rotary evaporator under reduced pressure and low temperature. The yield of each extract was weighed and stored in ambered bottles at 4 °C until used.

2. Preparation of Phytosomes: Accurately, weighed the quantity of lecithin (0.5 mg) and cholesterol (0.5 mg) was dissolved in a mixture of 5 ml of chloroform and 5 ml of acetone in the round bottom flask (RBF). Rotary evaporator (45-50 °C) is used to remove the organic solvent. After removal of solvent a thin layer of phospholipids was formed. This film was hydrated with ethanolic extract of the fruit of C. colocynthis in a rotary evaporator (37-40 °C for 1 h). Then prepared the buffer of 7.4 pH by using 0.8 gm of NaOH in 100 ml of water and 2.7 gm of KH₂PO₄ in 100 ml of water. Then the buffer was poured in the mixture of extract and phospholipids and rotated for 1 h in a rotary evaporator. Phytosomes were prepared. Then prepared phytosomes were filled in an amber colored bottle and stored in the freezer (2-8 °C) until used. All the protosomes were prepared by using the same method.

Characterization of Phytosomes:
Apparent Solubility: It was resolute by adding an excess of CC extract and prepared phytosomes to 5 ml of water or n-octanol in preserved glass containers at room temperature (25-30 °C). The liquids were agitated for 24 h then centrifuged for 20 min at 1,000 rpm to remove excessive CC extract or phytosome. The supernatant was filtered through a membrane filter (0.45 m). Then 1 ml filtrate was diluted with 9 ml of distilled water (H₂O), and absorbances were measured at 268 nm using UV spectrophotometer.

Entrapment Efficiency: Entrapment efficiency (EE) was calculated by using UV-visible spectrophotometer (UV-1601, Shimadzu). A measured quantity of phyto-phospholipid complex equivalent to 10 mg of CC extract was added to 50 ml methanol in a 100 ml beaker. The content was stirred on a magnetic stirrer for 4 h and then allowed to stand for 1 h. The clear liquid was poured out and centrifuged at 5000 rpm for 15 min. After that, the supernatant was filtered through
Whatman filter paper and following suitable dilution absorbance was measured in UV at 268 nm; to measure the concentration of the drug. The EE (%) was calculated using the following formula:  

\[ EE(\%) = \frac{T - S}{T} \times 100 \]

Where, T-Total concentrations of CC extract, S-is the CC extract contained in the filtrate.

**Particle Size Distribution:** The particle size examination of the prepared CC extract sample was conceded out using photon correlation spectroscopy, with dynamic light scattering on Zetasizer nano (Model: Nano series, S90 Zeta sizer, Malvern). The compound was discrete in isopropyl alcohol by stirring on a magnetic stirrer for 10 min. The dispersion was analyzed in Zetasizer.

**X-ray Diffraction (XRD) Study:** Diffractometer (Bruker, Germany) was used for the evaluation of the measurements of the samples. The operating conditions were current 0.8 mA; voltage 45 kV; scanning speed 1/min. The results were confirmed over a range of 5–60° (2θ) using the Cu-Anode X-ray tube and scintillation detector.

**Fourier Transform Infrared spectroscopy (FT-IR) Study:** FT-IR studies were performed on pure CC, Cholesterol, and dichloromethane extract of CC, ethanolic extract of CC, ethyl acetate extract of CC and aqueous extract of CC was in an Alpha FTIR spectrophotometer IR Affinity-1 (Shimadzu Corporation). A small amount of sample was positioned just under the probe on to which the probe was securely fixed, and then scanned the samples on the wave number region 4000-500 cm⁻¹. The attained IR spectra were interpreted for functional groups at their respective wave number (cm⁻¹).

**Scanning Electron Microscopy (SEM):** Phytosomal formulations were coated with gold in a Fine Coat Ion Sputter S-4800 TYPE II, Hitachi high technologies corporation, Japan. The analysis was made on the coated sample by introducing a pinch of sample in the S-4800 TYPE II (Hitachi high technologies corporation, Japan).

Scanning electron microscope (SEM) and surface morphology were viewed and photographed.

**Dissolution Study (in-vitro Drug Release):** The in-vitro dissolution summary of CC extracts and the prepared phytosomes were attained and compared. The studies were carried out in a USP XXIII, six station dissolution test apparatus, type II (VEEGO Model No. 6 DR, India) at 100 rpm and 37°C. An exactly weighed quantity of phytosome of CC extract (50 mg) was located into 900 ml of pH 6.8 phosphate buffer. Samples (3 ml each) of dissolution solution were withdrawn at different time intervals and then replaced with an equal amount of fresh standard to sustain sink conditions. Reserved samples were filtered (through a membrane filter), diluted properly, and then analyzed spectrophotometrically at 268 nm to establish drug release from the complex and the drug.

**RESULTS AND DISCUSSION:**

**Physico-chemical Characterization of Prepared Phytosomes:**

**Apparent Solubility:** The results of the measured apparent solubility of the CC extract and prepared phytosomes are shown in Table 1. CC extract had shown poor aqueous solubility (4 μg/mL), and comparatively elevated solubility in n-octanol (305 μg/mL), demonstrating a slightly lipophilic character of the drug. The physical mixture (PM), i.e., phytosome composite exposed a non-significant variation in the n-octanol solubility and a modest raise (1.5 times) in case of aqueous solubility. The prepared phytosome, though, illustrated a remarkable, and a considerable (over 12-fold) rise in the aqueous solubility. This rise in the solubility of the prepared complex might be explained by the partial amorphization, i.e., reduced molecular crystalline of the drug and the overall amphiphilic character of the phytosome.

**Particle Size Distribution:** The mean particle size of the phytosome having DCM extract of CC, phytosome having an ethanolic extract of CC, phytosome having ethyl acetate extract of CC and phytosome having an aqueous extract of CC was conceded out with dynamic light scattering technique. Surface area/volume (SA/V) ratio of the majority of particles is inversely proportional to the particle size. Thus, we conclude that the smaller particles of the prepared phytosomes, comprising a higher SA/V, that make it easier for the entrapped drug to be released out from the phytosome via
distribution and surface erosion. They also have the added benefit for the drug-entrapped phytosomes to pierce into and saturate through the physiological drug barriers. Previous studies showed that the smaller particles (≤500 nm) could cross the epithelial cell membrane via endocytosis, while the larger particles (≤5 mm) are taken up via the lymphatics. The mean particle size of phytosomal complex of DCM extract of CC was scattered in a narrow range of 254±20.0 nm, and the polydispersity index was 0.857±0.055.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Sample</th>
<th>Aqueous Solubility (µg/ ml)*</th>
<th>n-Octanol solubility (µg/ ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Citrullus colocynthis (CC)</td>
<td>4.45±0.33</td>
<td>3.565±0.54</td>
</tr>
<tr>
<td>2</td>
<td>Physical mixture (PM)</td>
<td>8.65±1.23</td>
<td>432.21±0.04</td>
</tr>
<tr>
<td>3</td>
<td>phytosomes of ethyl acetate extract of CC</td>
<td>85±0.09</td>
<td>617.34±0.58</td>
</tr>
<tr>
<td>4</td>
<td>phytosomes of ethanol extract of CC</td>
<td>76.4±0.65</td>
<td>564.85±0.29</td>
</tr>
<tr>
<td>5</td>
<td>phytosomes of aqueous extract of CC</td>
<td>62.7±0.56</td>
<td>635±0.32</td>
</tr>
<tr>
<td>6</td>
<td>phytosomes of dichloromethane extract of CC</td>
<td>89.4±0.04</td>
<td>725±0.51</td>
</tr>
</tbody>
</table>

X-ray Diffraction (XRD) Study: The X-ray diffraction (XRD) patterns of phytosomes of aqueous extract of Citrullus Colocynthis (1), phytosomes of ethyl acetate extract of Citrullus colocynthis (2), phytosomes of an ethanoloic extract of Citrullus colocynthis (3), phytosomes of dichloromethane extract of Citrullus colocynthis (4). The diffractogram of the 1, 2, 3 and 4 (shown in the figure below) revealed sharp crystalline peaks at 2θ=40.5°, 28° and 2.8°, 20=26°, 22.5° and 16°, 20=26°, 23°, and 16° and 20=46.5°, 31° and 24° respectively.

FT-IR Study: The results from the Fourier Transform Infrared Spectroscopy (FT-IR) analyses of pure CC, cholesterol, dichloromethane extract of CC, ethanolic extract of CC, ethyl acetate extract of CC and aqueous extract of CC were studied to get insight into the occurrence of interaction between extracts of CC and phospholipids. The FTIR spectrum of CC exhibited a broad peak at 3440 cm⁻¹, representing the aliphatic alcoholic (−OH) group, 1637 cm⁻¹ (Conjugated ester stretching), 1414-1558 cm⁻¹ (aromatic signals), 1400-1600 cm⁻¹.
(Aromatic absorptions stretching). FT-IR spectrum of cholesterol revealed the characteristic absorption at 2800-3000 cm\(^{-1}\) (asymmetric and symmetric stretching vibrations of -CH\(_2\) and -CH\(_3\) groups), 3442 cm\(^{-1}\) (-OH stretching), 1639 cm\(^{-1}\) (-C=O group), 1375 cm\(^{-1}\) (-CH\(_2\) and -CH\(_3\) bending vibration), 1055 cm\(^{-1}\) (ring deformation).

**FIG. 2:** A, SHOWS CRYSTALLINE STATE OF CITRULLUS COLOCYNTH, B, SHOWS PHYSICAL MIXTURE (PM), C, D, E, AND F SHOWS PHYTOSOMES OF ETHYL ACETATE EXTRACT OF CC, PHYTOSOMES OF ETHANOL EXTRACT OF CC, PHYTOSOMES OF AQUEOUS EXTRACT OF CC, PHYTOSOMES OF DICHLOROMETHANE EXTRACT OF CC

The FTIR spectrum of the prepared phytosomes from the different extracts of CC Fig. 2D is quite different from that of CC and cholesterol. The peaks at 2800-3000 cm\(^{-1}\) (asymmetric and symmetric stretching vibrations of -CH\(_2\) and -CH\(_3\) groups), 3315 cm\(^{-1}\) (attributed to -OH stretching), 1636 cm\(^{-1}\) (side chain carbonyl group) and 1389 cm\(^{-1}\) (-CH\(_2\) and -CH\(_3\) bending vibration) are shielded by phospholipids.

**FIG. 3:** FT-IR OF CHOLESTEROL

**FIG. 4:** FT-IR OF CITRULLUS COLOCYNTTHIS

The FTIR spectrum of the prepared phytosomes from the different extracts of CC Fig. 2D is quite different from that of CC and cholesterol. The peaks at 2800-3000 cm\(^{-1}\) (asymmetric and symmetric stretching vibrations of -CH\(_2\) and -CH\(_3\) groups), 3315 cm\(^{-1}\) (attributed to -OH stretching), 1636 cm\(^{-1}\) (side chain carbonyl group) and 1389 cm\(^{-1}\) (-CH\(_2\) and -CH\(_3\) bending vibration) are shielded by phospholipids.

**Percentage Drug Release:** The 12-h dissolution in the phosphate buffer (pH 6.8) exposed that, the pure CC showed the slowest rate of dissolution, because at the end of the dissolution period merely about 44% w/w of CC was suspended. The prepared phytosomes from the different extracts of CC, showed a significantly rapid release of CC at the end of the dissolution period, i.e., over 89% w/w of CC. It is cleared that the dissolution rate is influenced by the crystal morphology and the wet ability of the solids, and the enhanced dissolution rate explained by the enhanced solubility, and the moderately disrupted crystalline period (amorphous form) in the prepared complex. The moderately elevated amorphous state of the phytosome and their improved water-solubility may have a positive influence on the collective release of the drug.
CONCLUSION: Novel approach for herbal drug delivery is more prominent than conventional, which improves the bioavailability of polar extract and patient compliance. In the present study, an attempt was made to enhance the aqueous solubility of extracts of *C. colocynthis* via their complexation with phospholipids. The prepared phospholipid complexes were evaluated for physicochemical and functional attributes. The FTIR, SEM, and XRD study indicated the successful development of vesicular drug - phospholipids complex.

The apparent solubility and the in-vitro dissolution showed a significant improvement in the aqueous solubility, drug release, and membrane permeation. Supplementary studies for analyzing the pharmacokinetic parameters are required to validate the improved absorption and improved bioavailability hypothesis.

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