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FORMULATION DEVELOPMENT AND EVALUATION OF PHOSPHOLIPID COMPLEX OF SMILAX CHINA EXTRACT FOR EFFECTIVE TREATMENT OF DIABETES

Nirmla Tiwari*, Jagdish Chandra Rathi and Rahul Sharma

NRI Institute of Pharmaceutical Sciences, Bhopal (M. P.).

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***Corresponding Author**
Nirmla Tiwari
NRI Institute of Pharmaceutical Sciences, Bhopal (M. P.).

ABSTRACT

The powdered plant material (crude drug) contains active chemical constituents which are responsible for its biological activity. Extractive value determines the approximate measure of their chemical constituents in a given amount of plant material. % Yield of Pet. ether and Hydroalcoholic extract of *Smilax china* were found 2.58 and 5.76% respectively. In present study total phenol content was found 0.756mg/100mg, gallic acid equivalent in hydroalcoholic extract. Different formulation of phospholipids complex of hydroalcoholic extract of *Smilax china* Linn were prepared different ratio of phospholipids and cholesterol and evaluated for Microscopic observation of prepared Phytosome, Drug Excipient compatibility study by FT-IR, Entrapment efficiency, Particle size and size distribution, Transmission Electron Microscopy, *In-vitro* dissolution rate studies and Stability studies. The entrapment efficiency of the phospholipids complex was found in the range of 45.65±0.25 to 69.98±0.14%. Particle size of all formulations found within range 245.23±0.63-355.25±0.32nm. Acarbose at a concentration of (100-500µg/ml) showed α-amylase inhibitory activity from 51.19 to 88.75% with an IC50 value 35.33µg/ml and prepared Phospholipids complex showed the IC50 value of 444.63 µg/ml. This indicates that the Phospholipids complex of *Smilax china* Linn can be potent α amylase inhibitor in comparison with acarbose. stability studies clearly indicates that optimized batches of phospholipids complex were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content.

KEYWORD: Phospholipid Complex, *Smilax China*, Extract, Diabete.

INTRODUCTION

At current situation, human survival is largely dependent on the ecological resources on this planet.[1] Plant-based drugs have been used globally for human healthcare. In numerous zone of total population of world's remains relies upon (grown plants) herbal plants medicinal for their essential/primary health care needs especially where present day medicines are not available in their areas.[2]

Nano-formulations enhance the properties of conventional drugs and are specific to the targeted delivery site.[3] Phyto-phospholipid complexes are formed by interactions between active constituents and the polar head of phospholipids.[4] Interactions between active constituents and phospholipids enable phospholipid complexes to be an integral partin which the phospholipids head group is anchored, but the two long fatty acid chains do not participate in complex formation.[5] The two long fatty acid chains can move and encapsulate the polar part of complexes to form a lipophilic surface. Phyto-phospholipid complexes form agglomerates when diluted in water, which resemble a small cell that shows some similarity to liposomes; the differences between liposomes and complexes.[6] The active ingredient is distributed in the medium contained the cavity or in the layers of the membrane, whereas in phytosomes, it is an integral part of the membrane, being the molecules stabled through hydrogen bonds to the polar head of the phospholipids. Phospholipids are abundant in egg yolk and plant seeds.[7] Currently, industrially produced phospholipids are available.[8] A human biological membrane constitutes different classes of phospholipids, like phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidic acid (PA), and phosphatidylserine (PS).[9] PC possess two neutral tail groups and a positive head group which contains an oxygen atom in the phosphate group that has a strong tendency to gain electrons, while nitrogen to lose electrons, a rare molecular characteristic that makes PC miscible in both water and lipid environments.[10] Phyto-phospholipid complexes possess better drug complexation rate and the preparation of phyto-phospholipid complexes is not complicated.[11] In addition, phyto-phospholipid complexes show better stability trait because the formation of chemical bonds between plant extracts and phosphatidylcholine molecule.[12] By increasing the solubility of bile to active constituents, phyto-phospholipid complexes improve the liver targeting.[13]

Phospholipid complex-technique, can serve as a potent drug delivery system for increasing therapeutic index which encapsulates, plant actives.[14] In fact, these complexed actives are safer than its original form and can even serve as a better targeting agents to deliver these encapsulated agents at specific sites there by proving promising candidates in various medical fields for improving health aspects. This technique can be applied for herbal dosage form and are often known as phytosomes. Present investigation deals with formulate phospholipid complex of hydroalcoholic extract of *Smilax china* for effective anti-diabetic effect.

MATERIAL AND METHODS

Root of *Smilax china* L. was collected from local market of Bhopal the month of February, 2021.

Methods

Procurement of plant material: After the plant was collected they have been processed for cleaningin order to prevent the deterioration of phytochemicals present in plant. Soon after cleaning, plant material was kept for drying sun but under the shade. The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed.

Extraction procedure: The shade dried root of *Smilax china* was extraction with petroleum ether using maceration method. Root was extracted in hydroalcoholic solvent of (ethanol: water: 80:20). Powderedroot (62.4 gm) was extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in arefrigerator (4°C), till used for analysis.

Calculation of % yield

The % yield of yield of each extract was calculated by using formula:

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Preliminary phytochemical screening is primarily an important aspect for establishing

profile of given extract for its chemical compounds produced by plant. Phytochemical examinations were carried out extracts as per the following standardmethods.

Detection of alkaloids (Hager's test): Extracts dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates (Fehling's test): Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. 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.

Detection of glycosides (Legal's test): Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins (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 incidence of saponins.

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.

Detection of flavonoids (Lead acetate test): Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the occurrence of flavonoids.

Detection of proteins (Xanthoproteic test): The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

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 indicated the presenceof diterpenes.

Quantitative studies of phytoconstituents

Estimation of total phenol content

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method.

Preparation of standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various

aliquots of 10- 50µg/ml was prepared in methanol

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of Folin- Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortex for 15s and allowed to stand for 10min for color development. The absorbance was measured at 765 nm using ultra-violate spectrophotometer.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg ofextract.

Formulation development of phospholipids complex

Preparation of phospholipids complex

The complex was prepared with phospholipids: Cholesterol and *Smilax china* L. extract in the ratio of 1:1:1, 1:2:1, 2:1:1, 2:3:1 respectively. Weight amount of extract and phospholipids and cholesterol were placed in a 100ml round-bottom flask and 25ml of dichloromethane was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle.

Characterization of prepared phospholipids complex

Microscopic observation of prepared phospholipids complex: An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of

the optimized phospholipids complex formulation.

Drug Excipient compatibility study by FT-IR: IR spectra of physical mixture of drug and excipients were recorded by ATR (Attenuated total reflection) techniques using Fourier transform infrared spectrophotometer. A base line correction was made and the sample was directely mounted in IR compartment and scanned at wavelengths 4000 cm^{-1} to 400 cm^{-1}.

Entrapment efficiency: Phospholipids complex preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4. The clear supernatant was siphoned off carefully to separate the non entrapped phenol and the absorbance of supernatant for non entrapped extract of *Smilax china* L. Linn was recorded at λmax 765 nm using UV/visible spectrophotometer (Labindia 3000+). Sediment was treated with 1ml of 0.1 % Triton x 100 to lyse the vesicles and diluted to 100 ml with 0.1 N HCl and absorbance taken at 765 nm. Amount of quercetin in supernatant and sediment gave a total amount of extract of *Smilax china* L. in 1 ml dispersion. The percent entrapment was calculated by following formula.

$$\% \textbf{ Entrapment efficiency } = \frac{(\textbf{Drug added} - \textbf{free or unntraped drug})}{\textbf{Drug added}} \textbf{ X 100}$$

Particle Size and Size distribution: The particle size, size distribution and zeta potential of optimized phospholipids complex formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK) 95. The electric potential of the phospholipids complex, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.

Transmission electron microscopy: Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).

***In-vitro* dissolution rate studies**

In-vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.50C and 75 rpm. 10 mg of prepared phospholipids

complex was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (370C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy.

Mathematical treatment of *in-vitro* release data

The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

Zero order kinetics: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

Qt = Qo + Ko t

Where, Qt is the amount of drug dissolved in time t, Qo is the initial amount of drug in the solution (most times, Qo=0) and Ko is the zero order release constant.

First Order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

Where, Qt is the amount of drug dissolved in time t, Qo is the initial amount of drug in the solution and K1 is the zero order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

$$Q = K_H . t^{1/2}$$

Where, Q is the amount of drug released in time t and KH is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drugs.

Korsmeyer peppas model: Korsmeyer et al. used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\,t^n$$

Where, Mt/M∞ is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log Mt/M∞ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of n =0.5 for fickian diffusion and higher values of n, between 0.5 and 1.0, or n =1.0, for mass transfer following a non-fickian model. In case of a cylinder n =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent n the portion of the release curve where Mt/M□ < 0.6 should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (l) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and b value would be zero and only atn is used. This mathematical model, also known as Power Law, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms.

Stability studies of optimize phospholipids complex formulation

The prepared phospholipids complex subjected to stability studies at 40±2°C/75±5% RH and 30±2°C/60±5% RH as per ICH guidelines for a period of 3 months100. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance and drug content.

***In-vitro* anti diabetic activity of optimized formulation of phytosomes F-4**

Inhibition of alpha amylase enzyme

Preparation of standard: 10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100- 1000µg/ml were prepared in methanol.

Preparation of sample: 10 mg of dried phytosomes was extracted with 10 ml methanol. 500 µl of this extract solution was used for the estimation of enzyme inhibition.

Method: A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

Preparation of extract

Extract was prepared by maceration process

Extractive values

Root of *Smilax china* was extracted with hydroalcoholic solvent. Extractive values for petroleum ether was 2.58% and hydro alcoholic extract 5.76%. Extractive values are primarily useful for the determination of exhausted or adulterated drugs and it is an important tool to check the quality and variation in chemical constituents of the drug.

Phytochemical screening

Table 1: Result of phytochemical screening of smilax china.

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids (Hager's Test)	+ ve
2.	Glycosides (Legal's Test)	- ve
3.	Flavonoids (Lead acetate Test)	- ve
4.	Diterpenes (Copper acetate Test)	+ ve
5.	Phenol (Ferric Chloride Test)	+ ve
6.	Proteins (Xanthoproteic Test)	+ ve
7.	Carbohydrate(Fehling's Test)	+ ve
8.	Saponins (Froth Test)	- ve

Preformulation studies

Estimation of total Phenol and Alkaloid content

Total Phenolic content estimation (TPC)

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.019x + 0.020, R2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Calibration curve of gallic acid

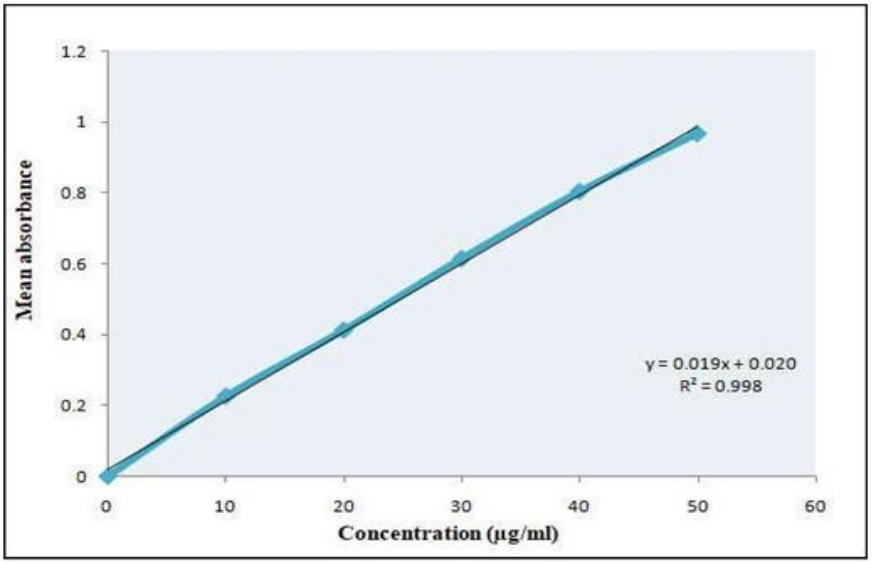

Figure 1: Graph of calibration curve of gallic acid.

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: y = 0.008x + 0.010, R2=0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

Calibration curve of atropine

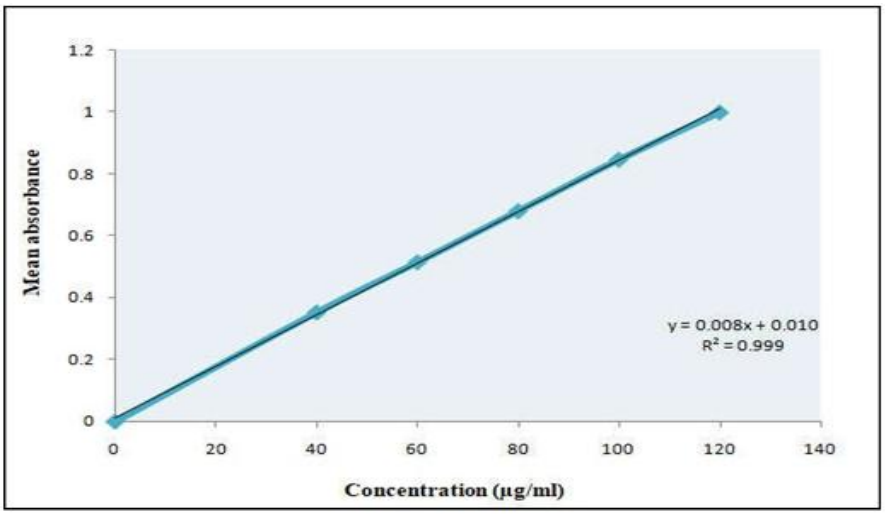

Figure 2: Graph of calibration curve of atropine.

Table 2: Estimation of total Phenolic and Alkaloid content of *smilax china.*

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.756	0.632

Characterization of phospholipids complex of hydroalcoholic extract of *smilax* china L.

Microscopic observation of prepared phospholipids complex

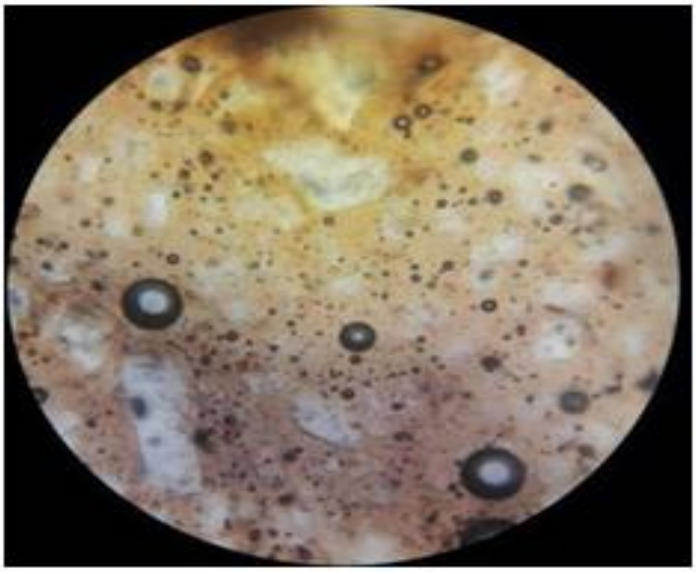

Figure 3: Microscopic observation of optimized batch F10.

Result of Drug-Excipient compatibility study

The spectrum of extract and phospholipids complex was authenticated by FTIR spectroscopy. The presences of characteristic peaks associated with specific structural characteristics of the

drug molecule were noted. Various peaks of the drug are shown in Figure and the wave numbers are listed in Table

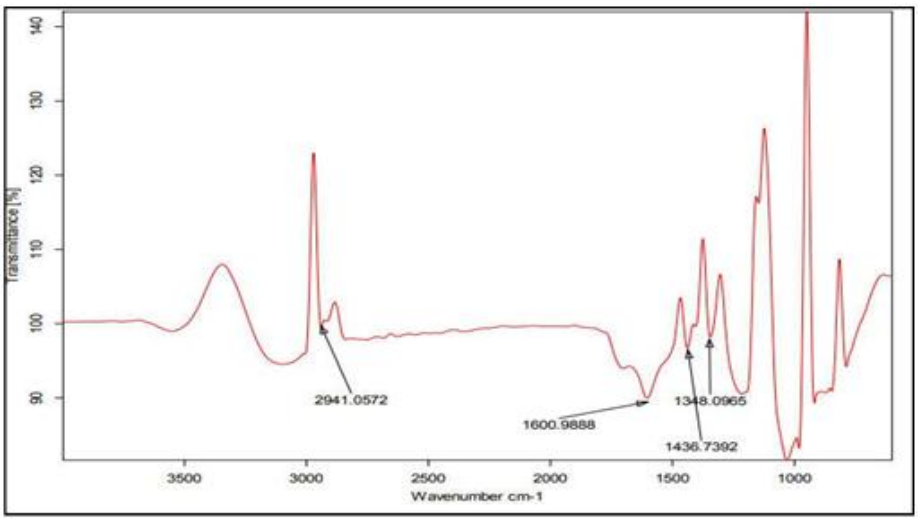

Figure 4: FT-IR spectra of hydroalcoholic extract.

Table 3: Interpretation of FT-IR spectrum.

S. No.	Functional group	Peak wave number(cm-1)	
		Experimental	Theoretical
1.	C-C str. and C=O str.	1600.9880	1550-1650
3.	O-H str.	2941.0572	3200-2800
5.	C=C aromatic str.	1436.7392	1400-1450

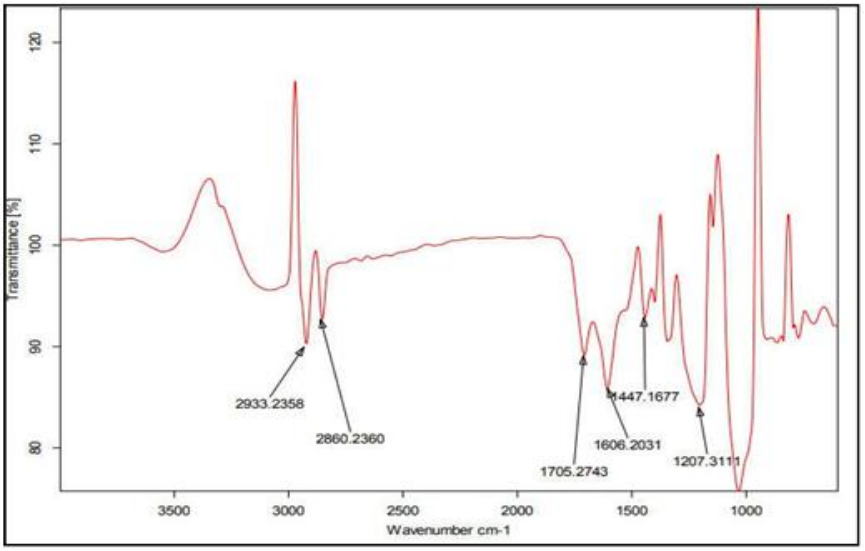

Figure 5: FT-IR spectra of prepared phospholipids complex formulation.

The appearance or disappearance of peaks and/or the shift of their positions are often indications of interactions such as hydrogen bonding. The IR spectra of extract, Fig. 7.6 7.7, shows stretching vibrations at 1600.9880 cm-1 attributed predominantly to the overlapping

stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Infrared of extract show stretching vibration at 2941.0572cm-1 due to O-H groups, C=C aromatic stretching vibration at 1436.7392cm-1. When the data obtained from FTIR spectra is compared with the spectra studied it was observed that there are similar peaks for functional groups in phospholipids complex.

From the FTIR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that during the process drug, Phospholipids and Cholesterol has not reacted with the drug to give rise to reactant products. So there is no interaction between them which is in favor to proceed for formulation of phospholipids complex as drug delivery system.

Entrapment Efficiency and Particle size analysis

Entrapment efficiency is an important parameter for characterizing phospholipids complex. In order to attain optimal encapsulation efficiency, several factors were varied, including the concentration of the lipid, concentration of drug and concentration of alcohol. The entrapment efficiency of all the prepared formulations is shown in Table 7.7. The entrapment efficiency of the phospholipids complex was found in the range of 45.65±0.25 to 69.98±0.14%.

Particle size of all formulations found within range 245.23±0.63-355.25±0.32nm. Concentration of lipid has shows significant impact on size of phospholipids complex. Formulation F4 was found best one which is further evaluated for drug release study, transmission electron microscopy (TEM), and stability studies.

Table 4: Particle Size and Entrapment efficiency of drug loaded phospholipids complex.

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	355.25±0.32	45.65±0.25
F2	325.58±0.45	48.85±0.45
F3	345.65±0.25	53.32±0.22
F4	245.23±0.63	69.98±0.14
F5	305.65±0.54	57.74±0.32
F6	298.85±0.47	55.65±0.65

Average of three determinations (n=3)

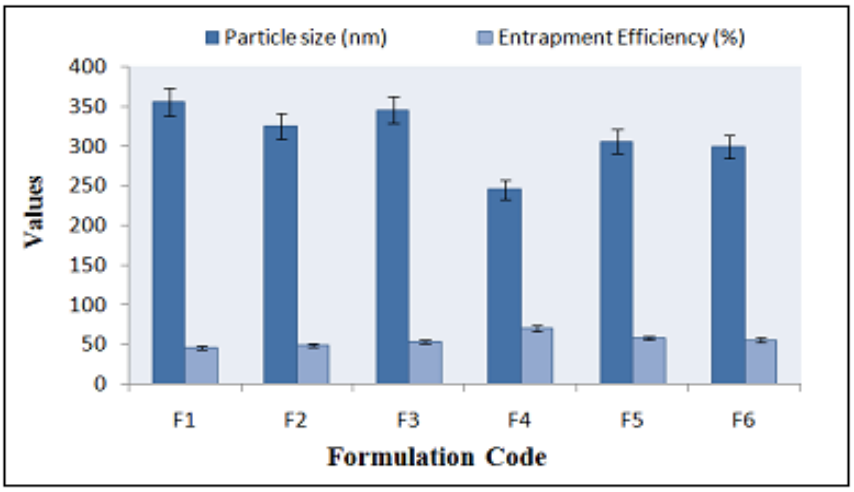

Figure 6: Graph of particle Size and Entrapment efficiency.

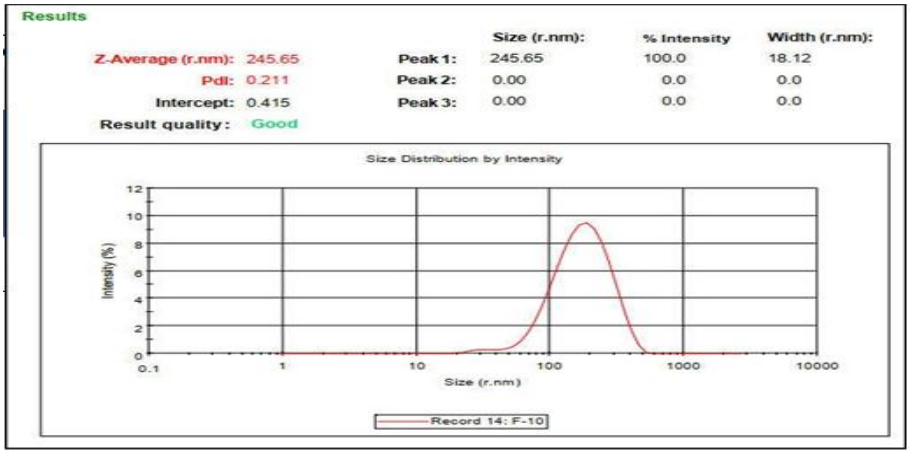

Figure 7: Particle size of optimized batch F4.

Transmission Electron Microscopy (TEM): TEM is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through.

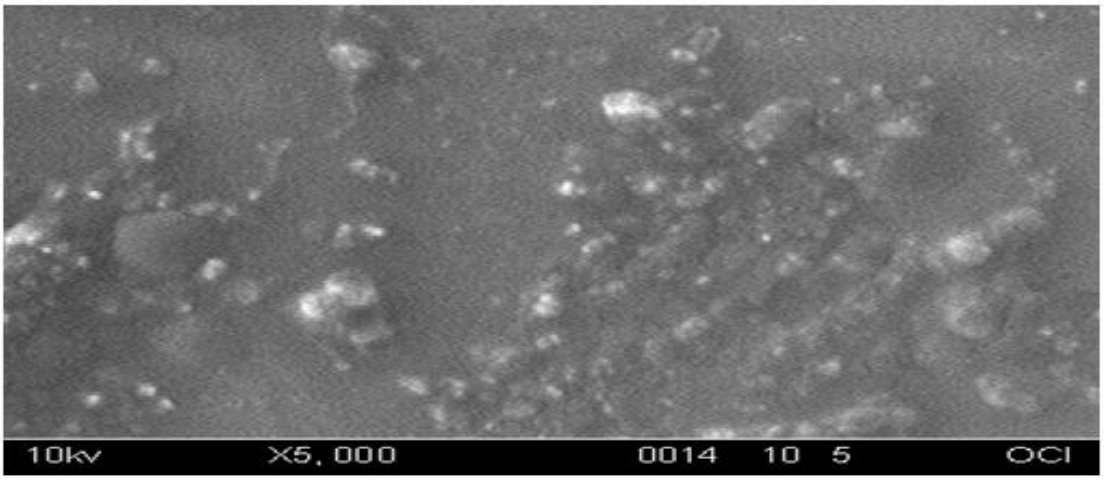

Figure 8: TEM image of phospholipids complex formulation F4.

***In-vitro* drug release study of prepared phospholipids complex formulation**

Table 5: In-vitro drug release data for optimized formulation F4.

Time (h)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	18.85	1.275	81.15	1.909
1	1	0	32.25	1.509	67.75	1.831
2	1.414	0.301	43.32	1.637	56.68	1.753
4	2	0.602	58.85	1.770	41.15	1.614
6	2.449	0.778	71.12	1.852	28.88	1.461
8	2.828	0.903	86.65	1.938	13.35	1.125
12	3.464	1.079	99.45	1.998	0.55	-0.260

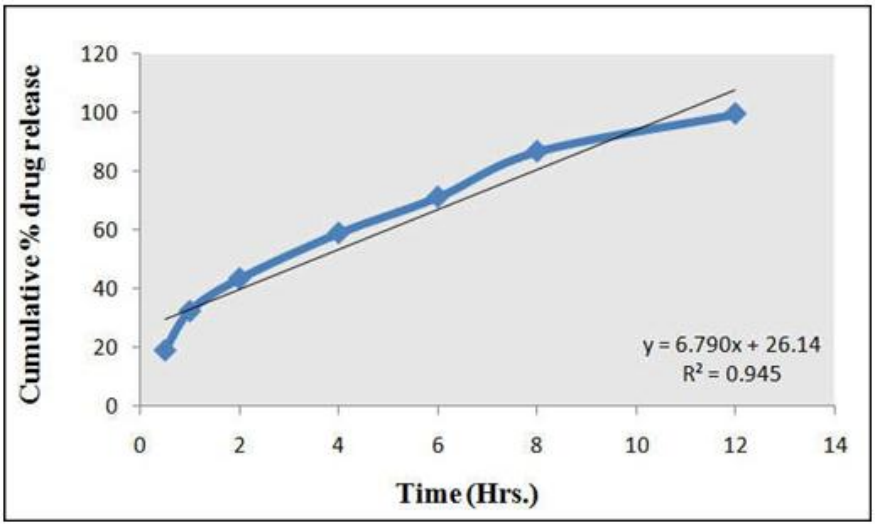

Figure 9: Cumulative % drug released Vs Time (Zero Order Kinetics).

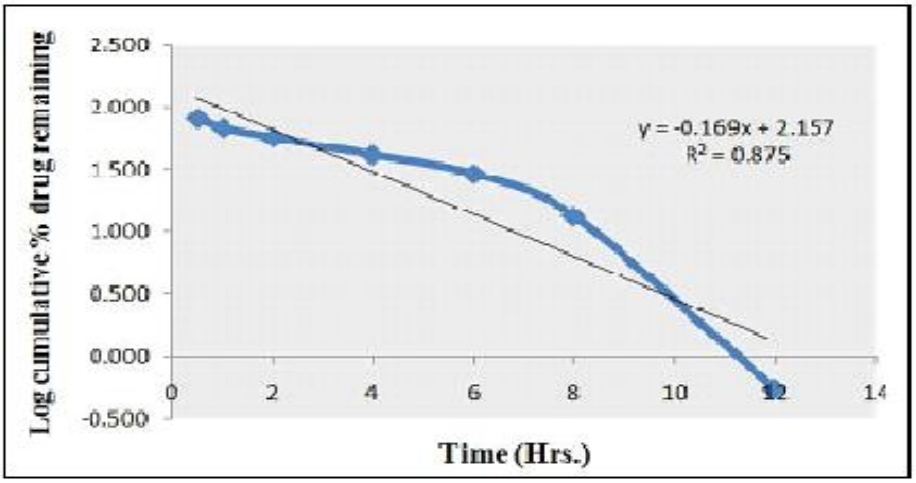

Figure 10: Log cumulative % drug remaining Vs Time (First Order Kinetics).

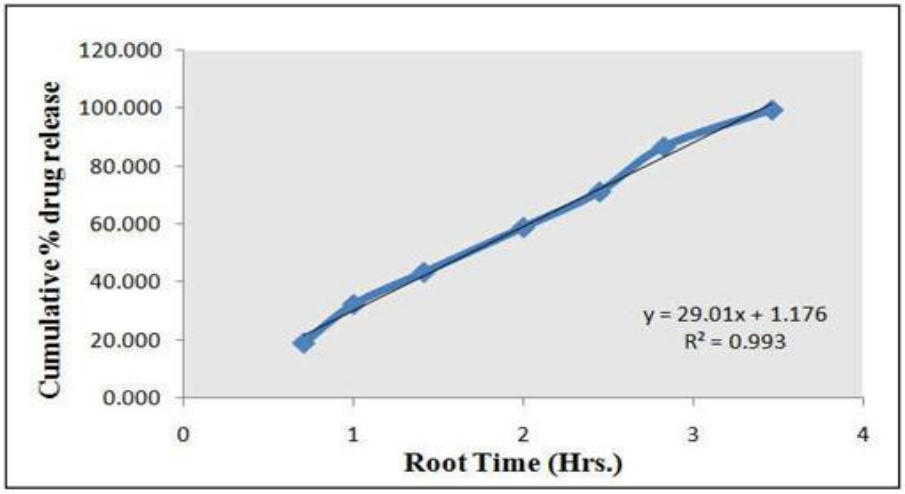

Figure 11: Cumulative % drug release Vs Root time (Higuchi release kinetics).

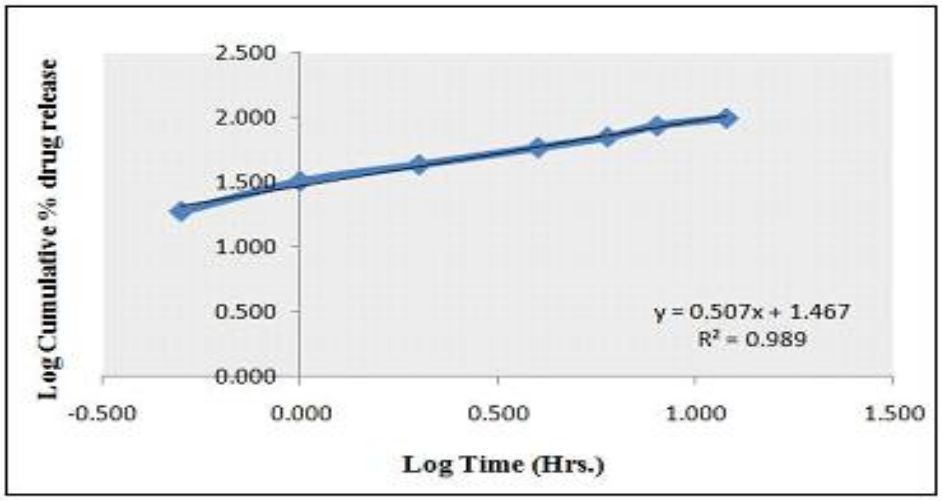

Figure 12: Log Cumulative % drug release Vs Log time (Korsmeyer Peppas Model).

Table 6: Regression analysis data of optimized formulation F4.

Batch	Zero Order	First Order	Higuchi	Korsmeyer- Peppas
	R^2	R^2	R^2	R^2
F4	0.945	0.875	0.993	0.989

When the regression coefficient values of were compared, it was observed that 'r2' values of Higuchi was maximum i.e. 0.993 hence indicating drug release from formulations was found to follow Higuchi release kinetics.

Stability studies

Results of stability studies clearly indicates that optimized batches of phospholipids complex were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content.

In-vitro anti diabetic activity of Phospholipids complex

Table 7: % Inhibition of Acarbose and Phospholipids complex F4.

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	Phospholipids Complex
1	100	51.19	27.54
2	200	70.10	33.65
3	300	74.20	39.14
4	400	85.18	42.88
5	500	88.75	57.69
IC_{50} (µg/ml)		35.33	444.63

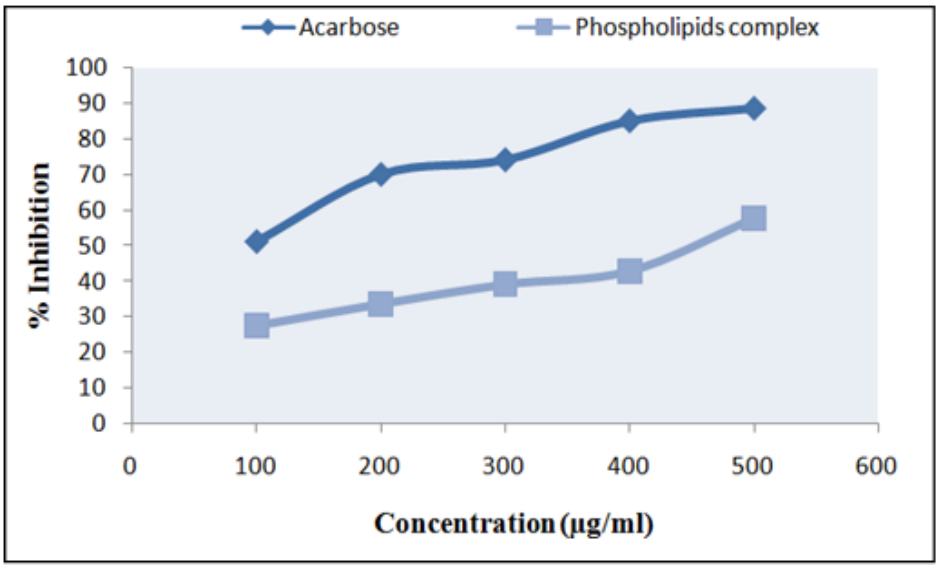

Figure 13: % Inhibition of Acarbose and Phospholipids complex formulation F4.

CONCLUSION

The powdered plant material (crude drug) contains active chemical constituents which are responsible for its biological activity. Extractive value determines the approximate measure of their chemical constituents in a given amount of plant material. % Yield of Pet. ether and Hydroalcoholic extract of Smilax china were found 2.58 and 5.76% respectively. Alkaloids, diterpenes proteins, carbohydrate, phenol, and saponins are essential secondary metabolites which are present in hydroalcoholic extract of Smilax china plant is summarized in Table 7.2. Except Flavonoids, glycosides, saponins and was absent. In present study total phenol content was found 0.756mg/100mg, gallic acid equivalent in hydroalcoholic extract. The result of levels of alkaloid in the tested extract was shown in Table 7.5. The concentration of alkaloid in crude extract 0.632mg/100mg Atropine equivalent in dry sample. Different formulation of phospholipids complex of hydroalcoholic extract of Smilax china Linn were prepared different ratio of phospholipids and cholesterol and evaluated for Microscopic observation of

prepared Phytosome, Drug Excipient compatibility study by FT-IR, Entrapment efficiency, Particle size and size distribution, Transmission Electron Microscopy, In-vitro dissolution rate studies and Stability studies. From the FTIR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that during the process drug, Phospholipids and Cholesterol has not reacted with the drug to give rise to reactant products. So there is no interaction between them which is in favor to proceed for formulation of phospholipids complex as drug delivery system.

Entrapment efficiency is an important parameter for characterizing phospholipids complex. In order to attain optimal encapsulation efficiency, several factors were varied, including the concentration of the lipid, concentration of drug and concentration of alcohol. The entrapment efficiency of all the prepared formulations is shown in Table 7.7. The entrapment efficiency of the phospholipids complex was found in the range of 45.65±0.25 to 69.98±0.14%. Particle size of all formulations found within range 245.23±0.63-355.25±0.32nm. Concentration of lipid has shows significant impact on size of phospholipids complex. Formulation F4 was found best one which is further evaluated for drug release study, transmission electron microscopy (TEM), and stability studies. When the regression coefficient values of were compared, it was observed that 'r2' values of Higuchi was maximum i.e. 0.993 hence indicating drug release from formulations was found to follow Higuchi release kinetics. Acarbose at a concentration of (100-500µg/ml) showed α-amylase inhibitory activity from 51.19 to 88.75% with an IC50 value 35.33µg/ml and prepared Phospholipids complex showed the IC50 value of 444.63 µg/ml. This indicates that the Phospholipids complex of Smilax china Linn can be potent α amylase inhibitor in comparison with acarbose. This could be justified that the nature of some extract constituents (Glycosides, phenols, flavonoids saponins, terpenoids) present in the extract could be responsible as being effective inhibitors of α-amylase.

Results of stability studies clearly indicates that optimized batches of phospholipids complex were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content. It can be concluded that the phospholipids complex containing Smilax china can provide a convenient and safe alternative to dosage form.

CONFLICTS OF INTEREST

There are no conflicts of interests.

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