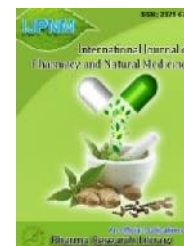




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RESEARCH ARTICLE

Design, development and characterization of phospholipid complex using *phyla nodiflora* extract

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ABSTRACT

The present study was carried out for phospholipid complexation with spray dried hydro alcoholic extract (SDHAE) of *Phyla nodiflora* Linn to improve oral solubility of the plant. Various chemical tests were performed on the extract and shown presence of various metabolites like amino acids, flavonoids, triterpenoids, glycosides, phenols, tannins etc. Plant SDHAE showed absorbance at 215 nm due to might be presence of triterpenoid Ursolic acid. The physicochemical investigations showed that the spray dried extract formed complex confirmed by FTIR, XRD and DSC with phosphatidylcholine. With reduced drug crystallinity and particle size, the phospholipid complex showed enhanced aqueous solubility. On the basis of the dissolution study & ex in-vivo absorption study, it might be concluded that the phospholipid complex may be considered as promising drug delivery system for improved absorption across the gastrointestinal tract for improving the bioavailability of the molecule.

Keywords : *Phyla nodiflora* extract, ursolic acid, Phosphatidyl choline complex.

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1. Introduction

The demand for the plant derived drugs seems to increase in developing countries due to their medicinal values and economic procurement. Modern herbal dosage form

includes tablet, capsule, soluble granule and ointment. As an advantage of modern herbal dosage form offers small dosage size and comparatively good absorption than

conventional dosage form. Modern dosage form is more flexible in carrying and can be taken even in a busy schedule hence play a tremendous role in clinical treatment. Even after advancement, modern herbal dosage forms still showing certain limitations such as delayed therapeutic response, lack of potential of reaching the drug to the target site, requirement of relatively large quantity of drug, chances of variability in herbals and destruction of the drug during its systemic passage from gastrointestinal tract (GIT) to liver, e.g. terpenoids. [1] Above mentioned limitations can be overcome by improving the therapeutic performance of established herbal drugs by formulating them in a new dosage form for the better drug delivery. [2] The plant *Phylla nodiflora* (Ratarilo, Family: Verbanaceae) is a slender prostrate, creeping, branched perennial herb with branches spreading profusely; branched fibrous roots; quadrangular stem with white hairs; opposite, sub-sessile, spatulate, deeply and sharply, appressed by two armed, white minute hairs on both sides of leaves; pink flowers and globose fruits with exalbuminous seeds. *Phylla nodiflora* is well known for its medicinal properties in Ayurveda and Unani systems of medicine for the treatment of burning sensation, anorexia, flatulence, colic, dyspepsia, worm infestations, diarrhoea, ulcers, asthma, bronchitis, knee joint pain, haemorrhoids, cardiac ailments, urinary retention, kidney disease, lymphadenitis, erysipelas and fever. [3] Researches in the past have established the genus *Phylla* to be the richest source of polyphenolic compounds especially triterpenoids, flavanoids, phenols, steroids like oleanolic acid, ursolic acid, amino acids, soluble sugar, starch, ascorbic acid, lipids, proteins nodiflorin-A, nodiflorin-B etc.

Terpenoids are the most important phytochemicals which modify the natural biological response due to its antiviral, anticancerous and anti-allergic properties but when these are administered orally or topically, have poor or very poor absorption. The reason for this poor absorption is due to the bacterial degradation of phenol moiety of the molecule or its complex formation with other substances present in the gastrointestinal tract (GIT). Therefore, development of novel delivery for this class of compounds are highly desirable in order to achieve improved bioavailability. Phospholipids are compound lipids derived from glycerol (Phosphoglycerides) or sphingosine (sphingomyelin). Being amphiphatic molecules, phospholipids in aqueous medium form minute, closed bilayered vesicles, which serve as permeability barriers. [4] Glycerol containing phospholipids have been put to wide range of applications. Their application is in medical device, liposomal drug delivery systems artificial blood and in diagnostic testing. [5,15] *Phylla nodiflora* phospholipid complex serves as novel drug delivery system consisting of microscopic vesicle that gives targeted and selective therapeutic response. It has improved pharmacokinetic and pharmacodynamic profile, which results in better pharmacological parameters in comparison to free form of drug and can be given by oral, topical, intra or

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extravascular routes form of drug and can be given by oral, topical, intra or extravascular routes.

2. Materials and Methods

Materials: Fresh aerial parts of *Phylla nodiflora* (Family: Verbanaceae) were collected during the month of September 2012 and authenticated by Dr. Alpesh M. Patel at Botany Department, J & J Science College, Nadiad. Herbarium specimen (DDU/FOP/12-13/S-04) submitted at Department of Pharmacognosy, Faculty of Pharmacy, DDU, Nadiad, Gujarat, India. Soya phospholipid was kindly provided by Ranbaxy Laboratories Ltd. (Indore, India). HPLC grade methanol, ethylacetate, diethylether and water were obtained from Merck (Mumbai, India). Dialysis membrane was procured from IPCA Laboratories Ltd. (Ratlam, India). The other chemical reagents were of analytical grade or better.

Methods

Extraction and preparation of plant powder:

Weighed 400 gm of dried powder of *Phylla nodiflora* was taken for an extraction in the round bottom flask. Methanol & water was used as the extraction solvent in ratio of 60:40. The flask placed for maceration over night. On the next day, exhaustive continuous reflux extraction carried out for 6 hours at 50 °C. Filter the extract and collect filtrate. Repeat such type of cycle's up to 7 days. All filtrate made concentrate up to one third of whole filtrate temperature not exceeding more than 60°C. Concentrated sample was filled into air tight container till spray drying carried out.

Characterization of complex:

For the prepared extract, chromatographic study was carried out by using various parameters like Particle size, solubility study, FTIR, DSC, XRD, in vitro drug dissolution study, ex vivo drug adsorption study using UV spectrophotometer to be performed for characterization. [8, 23-28]

Preparation of complex

Method I: The Spray Dried Extract Phospholipid complex was prepared by refluxing the SDE and phosphatidylcholine in (1:1) ratio. Both the reactants were placed in 100 ml round-bottomed flask containing 50 ml of dichloromethane. Prepared sample was ultra sonicated for 30 min. The reaction proceeded by refluxing the reaction mixture in a magnetic stirrer at 45–50 °C for 5 h. Thereafter, the volume of resulting solution concentrated to 2–3 ml, sufficient amount of n-hexane was added to get precipitation. The complex was collected, filtered, dried and stored in an air tight container until further use. [9]

Method II: A weighed amount of SDE and phospholipids in (1:1) ratio were charged in a round bottom flask and dissolved in anhydrous ethanol. Prepare sample was ultra-sonicated for 30 min. The mixture was refluxed at a controlled temperature for about 2 h. The resultant clear solution was evaporated and dried. The residues were then gathered and placed in desiccators. [10]

Particle Size: The particle size was determined by dynamic light scattering (DLS) using a computerized inspection system. [11]

Solubility Study: Solubility determination was carried out by adding an excess of sample to 5 ml of water in sealed

glass containers at 25°C. Each experiment was performed in triplicate. The liquids were agitated for 24 h and then centrifuged to remove solid substances (4000 RPM × 5 min). The supernatant was filtered through a 0.45 µm membrane. Then, 1 ml of filtrate was mixed with 9 ml of solvent, and a 10 µl aliquot of the resulting solution was measured in UV spectrophotometer.

Fourier Transform Infra-Red (FTIR):

The formation of the complex can be also be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectroscopy is also a useful tool for the control of the stability of complex when micro-dispersed in water or when incorporated in very simple cosmetic gels. From a practical point of view, the stability can be confirmed by comparing the spectrum of the complex in the solid form.^[12]

Differential scanning calorimetry (DSC):

Differential scanning calorimetry (DSC) is a fast and reliable method to detect drug-excipient compatibility to provide maximum information regarding the possible interactions. An interaction is concluded by the elimination of endothermic peaks, appearance of new peaks and change in peak shape and its onset, peak temperature/melting point, and relative peak area or enthalpy. DSC thermo grams of SDE, phospholipid and SDE - Phospholipid complex were recorded using differential scanning calorimeter. The thermal behavior was studied by heating 2.0 ± 0.2 mg of each individual sample in a covered sample pan under nitrogen gas flow. The investigation was carried out over the temperature range 0–300 °C at a heating rate of 10 °C min⁻¹. It is fast and reliable method to identify the formation of phospholipid complexes.^[13]

X-ray powder diffraction (XRD):

The X-ray diffraction was recorded on an X-ray Diffractometer. Samples of certain weight were added into the slide for packing prior to X-ray scanning. Spectra of graphs were plotted from 5.00° to 65.00° of 2 θ with a step width of 0.03° and step time of 0.2 s at room temperature.

In Vitro drug dissolution study:

The in vitro release of SDE- Phospholipid complex was investigated in eight station dissolution test apparatus compared to SDE of plant with a USP apparatus II using phosphate buffer (pH 7.2) at 75 rpm and at 37 °C. An accurately weighed amount of the complex equivalent to 100 mg of drug was put into 900 ml phosphate buffer (pH 7.2) media. Samples (5 ml each) of dissolution fluid were withdrawn at 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240 min. and replaced with the equal volume of fresh media, to maintain sink conditions. Withdrawn samples was filtered (through a 0.45 mm membrane filter) and diluted suitably and then analyzed spectrophotometrically.^[14]

Ex Vivo Absorption Study Using Everted Small Intestine Sac Method: The fresh intestine of sheep was brought from the local butcher's shop in transport buffer within half an hour of slaughtering of the animal.^[15]

Composition of transport buffer:

Name of Ingredient Quantity

Calcium chloride :0.1324 g

Magnesium chloride :0.2438 g

Disodium hydrogen phosphate :0.3406 g

Sodium di hydrogen phosphate :0.0624 g

Sodium bicarbonate :2.1002 g

Potassium chloride :0.3726 g

Sodium chloride : 6.6716 g

Glucose : 0.8970 g

Distilled water : Required to produce 900 ml

After discarding approx. 10–15 cm section from the pyloric end intestine (7–9 cm.) was taken, washed and freed of intestinal contents, and everted using glass rod. The everted gut was stretched under a weight of 10 gm. A weight of 10 gm was attached to the ligated end to keep the sac in a vertical position. Prepared sac was placed in a beaker containing 400 ml PBS (7.2 pH) solution as well as dispersed complex. Continuous aeration was provided during the procedure. Then 2 ml of PBS was introduced into sac from cannula. After time interval of 20, 40, 60, 90, 120, 150, 180, 210, 240 min. 1 ml sample was withdrawn with replacing 2 ml fresh media. All samples were analyzed by spectrophotometrically with required dilution.

3. Results and Discussion

Extraction of plant *Phyla nodiflora* was done by using Hydro alcoholic solvent, methanol:water (60:40 ratio). Continuous reflux extraction was carried out. After drying of concentrated filtrate 18.24 gm spray dried powder was obtained and stored in air tight container.

Chemical tests

Various chemical tests were performed on hydro alcoholic extract of plant. This shows presence of various primary metabolites like amino acids and proteins in the hydro alcoholic extract of plant. Tests also show presence of secondary metabolites like flavonoids, triterpenoids, glycosides, phenols, tannins etc, in the hydro alcoholic extract of plant.

Absorbance of SDE of *Phyla nodiflora* in Methanol:

A weighed amount of SDE was taken and stock solution in methanol was prepared. A peak was observed at the 215 nm. That might be due to present of Ursolic acid in the SDE. Because Ursolic acid having absorbance between range of 210 -218 nm. So from the stock solution dilution pattern was prepared and absorbance was taken at 215 nm. It shows linearity in the peak. Calibration curve was prepared from the absorbance taken at 215 nm. Linear regression of absorbance on concentration gave equation $y = 0.117x - 0.1497$ with a correlation coefficient of 0.991.

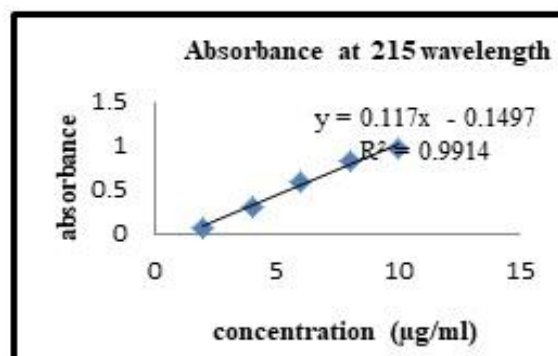


Figure 1:(A) Beers plot (Plot of Conc. vs. Absorbance)

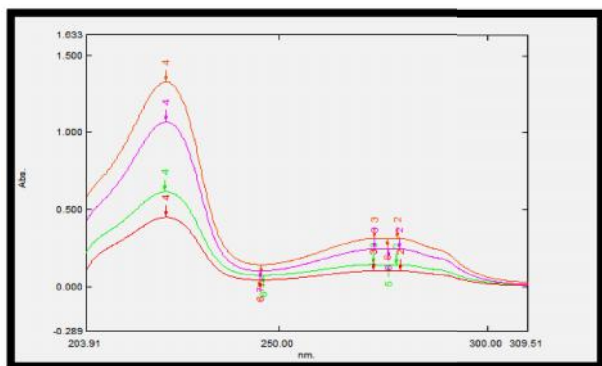


Figure 1:UV spectrum of SDE of *Pnodiflora L.* in Methanol

Preparation of Spray Dried Extract Phospholipid Complex:

Phospholipid complex prepared by using two different phospholipids 90 G and 90 H as well as using two different solvents Dichloromethane and Ethanol. Percentage yield obtained from different complexes shown in table. In complex 2 & 4 more practical yield was obtained. So ultimately complex prepared with Phospholipon shows higher practical yield than complex prepared with Phospholipon 90 G.

Characterization of Phospholipid Complex:

Particle size: Particle size of SDE and all prepared complex was determined by using Malvern dynamic light scattering instrument at Shah-Schulman Centre for Surface Science and Nanotechnology, D.D.University, Nadiad.

Particle size of SDE and phospholipid complex:

Particle size analysis shows that SDE having particle size near about 1000 d.nm while all prepared complex shows particle size in the range of 531 – 619 d.nm except UPC4 shows particle size 982 d.nm. That indicate the complexation of SDE with phospholipid reduce the particle size of SDE.

Solubility Study: Aqueous solubility of SDE and prepared complex was carried out in water. Samples were analyzed using spectrophotometer. Results shows at some extent aqueous solubility was increased of prepared complex as compared to SDE.

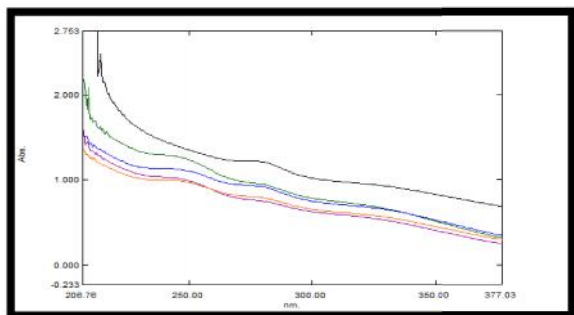


Figure 3:UV spectra of SDE and SDE-Phospholipid complexes

Aqueous solubility study of SDE and phospholipid complex: All phospholipid complex complex showed good aqueous solubility in hydrophilic medium (in water) and found to be higher than the free SDE. SDE of plant is poorly miscible in aqueous media, but the complexation

with the phospholipid increased the solubility of SDE in water. UPC 1 & 3 shows more aqueous solubility then complex UPC 2 & 4.It might be due to complexation of SDE with Phospholipon 90 G. This increase in solubility of the complex may be explained by the amorphous characteristics of the complex (due to reduced molecular crystallinity of the drug) and amphiphilic nature of the complex. The complex showed an amphiphilic nature, which in turn may result in improved absorption across the GIT (gastrointestinal tract) for improved availability of the SDE in the systemic circulation.

FTIR: The formation of the complex can be also be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures.

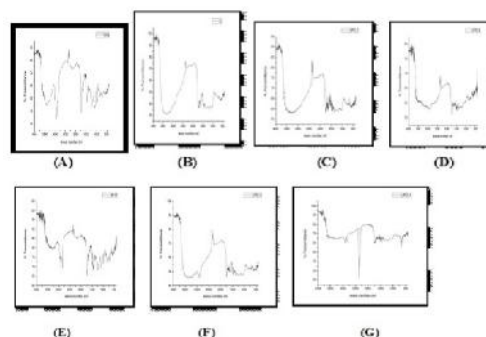


Figure 4:Infrared emission spectra over the wavelength range 400–4000 cm-1 (A) Phospholipon 90 G, (B) SDE, (C) UPC 1, (D) UPC 3, (E) Phospholipon 90 H, (F) UPC 2, (G)UPC 4

FTIR spectra shows peak of OH banding at 3600-3200, COOH-2400, CH3 1470-1350 in the spectra of Phospholipon 90 G with spectra of SDE and Phospholipon 90 H with spectra of SDE. These all peaks were also observed in the spectra of UPC 1 and UPC 3. So there is no chemical incompatibility observed in FTIR spectra. The overall observation indicated that phenol groups of SDE have participated in the complex formation with the polar head of the phosphatidylcholine molecule.

Differential scanning calorimetry (DSC):

Differential scanning calorimetry study of SDE and prepared complex was carried out over the temperature range 0–300 °C at a heating rate of 10 °C min⁻¹.

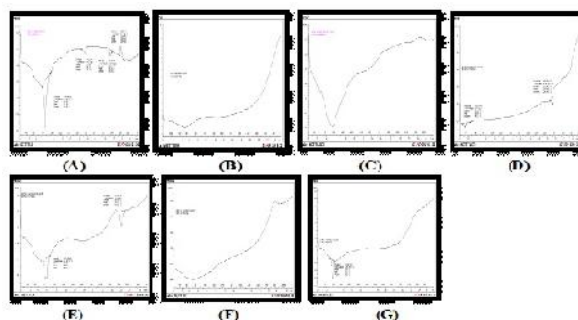


Figure 5: DSC curve of (A) Phospholipon 90 G, (B) Phospholipon 90H0, (C) SDE(D) UPC 1, (E) UPC 2, (F) UPC 3, (G) UPC 4

It is a fast and reliable method to detect drug-excipient compatibility to provide maximum information regarding the possible interactions. An interaction is concluded by the elimination of endothermic peaks, appearance of new peaks, and change in peak shape and its onset, peak temperature/melting point, and relative peak area or enthalpy. From the Figure, the Crystals of SDE show the endothermic peak at about 80 °C, corresponding to the product melting and the phosphatidylcholine exhibited a typical endothermic peak at about 86 °C.

Prepared all phospholipid complex showed complete disappearance of the endothermic peaks of the individual component and exhibited a broad new peak at about 70.22 °C in the DSC curve and supported the interaction of SDE molecule with the phospholipon 90 G & 90 H.

X-ray powder diffraction (XRD):

In the X-ray diffractogram of SDE don't showed intense diffraction peaks of crystallinity at a diffraction angle of 2theta and suggested that the material may present as an amorphous material. The phospholipon 90 H showed a single diffraction peak. A total drug amorphization was induced by the complex formation where X-ray diffraction pattern of the SDE-Phospholipid complex was characterized only by large diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of the SDE. UPC 1 & UPC 3 shows reduced sharp peak as compared to the individual. UPC2 & UPC 4 also shows one sharp reduced peak. So it can be conclude that molecule of SDE goes to amorphous state by complexation with phospholipid then the pure SDE.

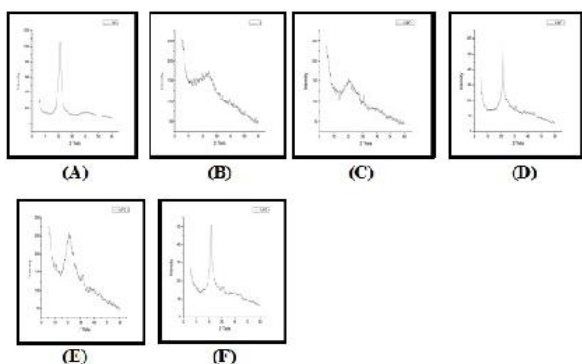


Figure 6:X-ray diffraction patterns, (A) Phospholipon 90 H (B) SDE(C) UPC 1 (D) UPC 2 (E) UPC 3 (F) UPC 4

In vitro drug dissolution study: In vitro drug dissolution study of SDE and prepared extract was performed using USP apparatus II. A media was used Phosphate buffer of pH 7.2, at 75 rpm and at 37 °C. Obtained results of dissolution data of % cumulative release of SDE and phospholipid complex listed in table.

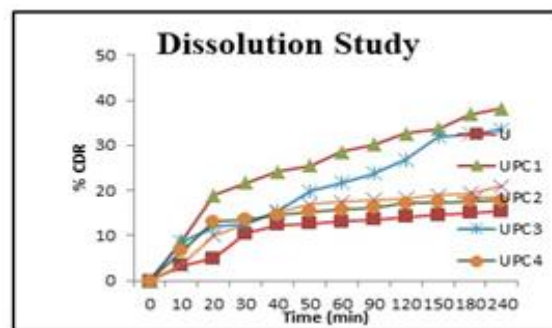


Figure 7: In-vitro drug dissolution study, %CDR vs. Time (min)

In vitro drug dissolution study of SDE and all phospholipid complexes shows that, after 210 min SDE release 15.39 % CDR. In other all complexes it was increased as compared to SDE. UPC 1 shows 38.16%, UPC 2 shows 20.94%, UPC 3 shows 37.57%, and UPC 4 shows 17.77% CDR. UPC 1 & UPC 3 shows higher release might be due to Phospholipon 90 G. So from the dissolution study it can be concluded that Phospholipon 90 G provides higher drug dissolution than Phospholipon 90 H, so ultimately Phospholipon 90 G improves solubility of SDE.

Ex Vivo Absorption Study Using Everted Small Intestine Sac Method: Ex in-vivo absorption study by using everted small intestine sac method. Fresh small intestine of sheep was used for study. From the time interval sample was withdrawn, diluted and analyzed spectrophotometrically.

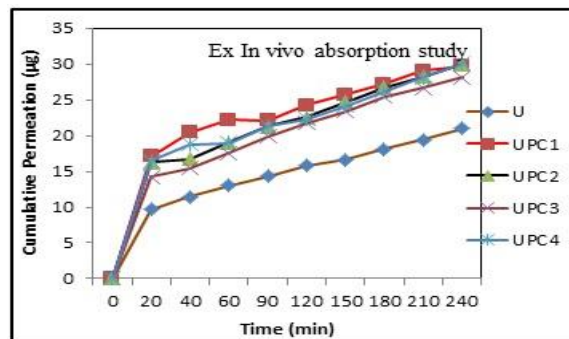


Figure 8:Ex in vivo absorption study, cumulative permeation (µg) vs. Time (min)

Absorption of SDE and all prepared complexes was investigated. Study shows absorption of SDE- 21.02 µg, UPC1-29.75 µg, UPC 2-29.96 µg, UPC 3-28.17 µg, UPC 4-30.5 µg after 240 min. The curve between cumulative permeation and time for SDE and SDE-PC complex is shown which clearly indicates the enhanced absorption of SDE-PC complex.

Table 1:Complex coding and specification

Complex	Method Used	Phospholipid	Solvent
UPC 1	1	90 G	Dichloromethane & n-Hexane
UPC 2	1	90 H	Dichloromethane & n-Hexane
UPC 3	2	90 G	Ethanol
UPC 4	2	90 H	Ethanol

Table 2: Extraction of *Phyla nodiflora* by continuous reflux extraction method

Extract	Plant Part	Weight of powder taken	Practical Yield	% Yield (w/w)
Methanol:Water 60:40	Whole plant	400 gm shade dried powder of plant	18.24 gm dried powder obtained after spray drying of concentrated extract	4.57%

Table 3: Chemical constituent present in hydro alcoholic extract of *Phyla nodiflora* Linn

Constituents	Identification test	Hydro alcoholic extract
Flavonoids	Shinoda test	-
	With lead acetate	+
Alkaloids	Dragendroff's reagent	+
	Mayer's test	-
Proteins and free Amino acids	Million's reagent:	+
	Biuret's test:	+
Carbohydrates	Fehling's test	-
	Molisch's test	-
Saponin	Foam test	+
Glycoside	Borntrager test	+
Tannins	5% FeCl ₃ solution	+
Triterpenoids	Salkowski test	+

Table 4: Absorbance of SDE of *Phyla nodiflora* in Methanol

Conc. (µg/ml)	Absorbance			Average ±SD
	I	II	III	
2	0.059	0.072	0.064	0.065 ± 0.006
4	0.297	0.317	0.308	0.307±0.010
6	0.577	0.602	0.593	0.591±0.012
8	0.806	0.834	0.817	0.819±0.014
10	0.966	0.992	0.979	0.979±0.013

Table 5: Practical yield of prepared SDE-Phospholipid complex

Complex	% Yield(W/W)
UPC 1	53.93
UPC 2	66.55
UPC 3	56.62
UPC 4	61.16

Table 6: Particle size of SDE and phospholipid complex

Sample Name	Particle Size (d.nm)	% Intensity
Extract	1003	47%
UPC 1	547	100%
UPC 2	619	100%
UPC 3	531	85%
UPC 4	982	77%

Table 7: Aqueous solubility study of SDE and phospholipid complex

Sample Name	Aqueous solubility (µg/ ml)
Extract	15.72
UPC 1	24.18
UPC 2	17.06
UPC 3	23.60
UPC 4	16.61

Table 8: In vitro drug dissolution study

Time (min)	%CDR				
	U	UPC1	UPC2	UPC3	UPC4
0	0	0	0	0	0
10	3.37	8.90	3.48	8.56	6.48
20	4.92	18.86	9.98	11.89	13.14
30	10.47	21.67	12.70	12.66	13.49
40	12.31	24.15	15.16	15.43	14.52
50	12.71	25.44	16.84	19.76	15.21
60	13.11	28.53	17.46	21.72	15.74
90	13.50	30.21	17.88	23.68	16.23
120	14.05	32.59	18.22	26.74	17.12
150	14.52	33.69	18.98	31.96	17.35
180	14.97	36.81	19.36	32.44	17.60
210	15.39	38.16	20.94	37.57	17.77

Table 9: Ex In vivo absorption data

TIME	U	UPC 1	UPC 2	UPC 3	UPC 4
0	0	0	0	0	0
20	9.660256	17.11526	16.29026	14.26026	16.47526
40	11.39231	20.44831	16.71331	15.40731	18.72031
60	13.03436	22.17836	19.02936	17.58836	18.97036
90	14.32641	22.08341	21.23041	19.90341	21.27841
120	15.75846	24.25046	22.58346	21.73246	22.32946
150	16.63051	25.70351	24.68551	23.40851	24.16051
180	18.14256	27.16556	26.77856	25.47456	26.16456
210	19.45462	29.03562	28.25362	26.68862	28.13562
240	21.02667	29.75467	29.96867	28.17467	30.10167

4. Conclusion

The present study was carried out for phospholipid complexation with spray dried extract of *Phyllanthus nodiflora* Linn to improve oral solubility of the plant. The study of extraction of plant with hydro alcoholic solvent results in good practical yield of extract. Hydro alcoholic extract of plant was spray dried and analytical estimation was done. Plant SDE shows absorbance at 215 nm due to might be presence of triterpenoid Ursolic acid. The physicochemical investigations showed that the spray dried extract formed complex confirmed by FTIR, XRD and DSC with phosphatidylcholine. With reduced drug crystallinity and particle size the phospholipid complex showed enhanced solubility. Complex UPC 1 & 3 shows higher reduction in particle size then the SDE ultimately improves aqueous solubility of the SDE. On the basis of the dissolution study & ex in-vivo absorption study, it may be concluded that the phospholipid complex may be considered as promising drug delivery system for improved absorption across the gastrointestinal tract for improving the bioavailability of the molecule.

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