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Formulation and evaluation of *Ziziphus xylopyrus* loaded phytosomes

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Abstract
The leaves of *Ziziphus xylopyrus* having traditionally use as treatment of diabetes and hepatoprotective activity. The *Ziziphus xylopyrus* has limited gastrointestinal absorption because of its low bioavailability. Different flavonoids are slowly absorbed because the multi-ring compound. Many flavonoids they can cross lipid rich outer membrane of small intestine.
The methanolic extract of phytosome was prepared in soya lecithin. Soya lecithin has antioxidant activity. Evaluation of phytosome for solubility study, Entrapment efficiency Transition temperature, X-ray diffraction, *In vitro* dissolution studies, FTIR study, an *in vivo anti-diabetic* activity of soya lecithin and *Ziziphus xylopyrus* can result in synergistic effect. Formulation of phytosome they can be loaded into capsule dosage form.

Keywords: antidiabetic, hepatoprotective, *Ziziphus xylopyrus*, phytosome, bioavailability, soya lecithin

Introduction
Diabetes mellitus may be defined as a persistent illness characterized by high blood glucose level due to absolute or relative deficiency of circulating insulin level or insulin resistance. Though different type of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use the natural products with Antidiabetic activity to overcome the side effects and toxicity of synthetic drugs. Home grown medications are endorsed broadly as a result of their viability, less symptoms and minimal effort. The aim of present work is to confirm the effect of Antidiabetic extracts of *Ziziphus xylopyrus* and to evaluate the Hypolipidemic and antioxidant potential of these extracts in STZ diabetic rats.
Ziziphus xylopyrus family Rhamnaceae is usually known as Jujab in English [1]. It is a huge, straying bush or a little tree, outfitted with spines, up to 4 m in tallness. Natural products are globose, 3-seldom 2 or 4-celled, with typically a seed in every phone, extremely hard and woody. It is found in Pakistan and China, North-Western India, Uttar Pradesh, Bihar, Central and South India [2]. The natural product decoction of this plant is utilized in anti-conception medication in certain pieces of Rajasthan, India [3]. This plant is generally utilized in Turkish medication as a powerful sedative [4]. Bark and leaf powder glue are applied remotely for chest torment emerging from hack. In stomach throb and acid reflux, organic product powder (3-4 g) is controlled with a touch of ginger powder threefold in a day. The leaves contain quercetin and quecitrin. The bark contains tannins (7.2%) 7, 3, 4-trihydroxy flavan-3, 4-diol and oleanic acid [5]. *Z. xylopyrus* is utilized in Pyorrhoea and to check oogenesis [6]. The bark is utilized for its astringent action and as dental sticks for teeth cleaning. In various pieces of India this plant is likewise utilized in the treatment of diarrhoea [7]. Leaf glue is applied on pimples and it is ground alongside latex of *Ipomea carnea* and applied on boils [8]. One to two crawls of the new stem bark of this species are bitten with 1-2 peppers (*Piper nigrum*) and the sap gulped once per day for 5 days in the treatment of cough [9]. Xylopyrine-An and Xylopyrine-B, the two new 13-membered cyclopeptide alkaloids are additionally present in Ziziphus species for example *Z. xylopyra* [10]. The plant is reported to contain alkaloid jubanine-E [9]. It contains three flavones-C-glucosides-6”sinapoylspinosin, 6”-feruloylspinisin and 6-“p-coumaroylspinosin. The leaves and stems of *ziziyphus jujuba* lam contain saponins 3-o-[2-α-L-fucopyrnosyl-3-o-β-D-glucopyranosyl-α-L-arabinopyranosyl jujubogenin. The fruits of *Zizyphus jujuba* lamk contain zizyphus saponins I, II, III and jujuboside B [11], jujuboside D [12], and jujuboside e [13]. The bark of *Zizyphus jujuba* Lamk contains 7% tannin [14]. Thus, it were decided that in present study, formulation of phytosomes containing leaves extract of *Ziziphus xylopyrus and soya lecithin* and evaluated for anti-diabetic potential.

Materials & Methods
Materials
The *Ziziphus xylopyrus* leave extract was purchased from the AMSAR Pvt. Ltd., M.P, India.
All the other chemicals and reagents used in this study were of AR grade and were purchased from SD Fine chemicals, Mumbai.

Instruments
Schemadzu UV-vis spectrophotometry model 1800, laboratory, Perkin Emer Spectrum 68 FTIR, Dissolution tester (USP) TDT-08L (Electrolab).

Formulation of Phytosomes

Solvent Evaporation Technique [7]

10 g of soya lecithin was broken down in 25 ml of chloroform. The blend was refluxed under mechanical stirrer include drop insightful include 10gm of methanolic portion of aq. concentrate of *Ziziphus xylopyrus* arranged by including 70 ml of methanol kept on a mechanical stirrer. The mixture was refluxed under stirring for 5-6 hour, then concentrated and finally dried under vacuum at 40°C for 48 hours. The resultant *Ziziphus xylopyrus* phospholipid complex was kept in an amber colored glass bottle and stored for refrigerator.

Evaluation of Phytosome [8-10]

The phytosomes formulation was evaluated by following testes

1. Solubility study.
2. Entrapment efficiency.
3. Transition temperature (DSC).
4. X-ray diffraction of formulation.
5. *In vitro* dissolution studies.
6. Fourier transforms infrared (FTIR) study to check stability.
7. *In vitro* radical scavenging activity of phytosomes by DPPH model.

a. Solubility Study [8]

10 ml of solvent in glass containers at room temperature. The liquid was agitated for 24 hours on rotator shaker then centrifuged for 15 minutes to remove excessive *Ziziphus xylopyrus* extract. The supernatant was separated through layer channel. At that point 1 ml of filtrate was blended in with 9 ml of methanol to get ready weakening and these samples were estimated at frequency of 271 nm by UV spectrophotometer. The absorbance of focus was determined by utilizing alignment bend.

b. Drug Entrapment Efficiency [9]

100 mg of pure *Ziziphus xylopyrus* extract in methanol. Extract was centrifuged for 40 min at 24°C to separate the drug in the supernatant from the drug incorporated in the phytosomes. Concentrations of *Ziziphus xylopyrus* extract in the supernatant were determined by UV-visible spectrometry at 271 nm. The entrapment efficiency was calculated according to the following equation:

$$\text{Percentage Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total Drug}} \text{ X } 100$$

c. Transition temperature [9]

The transition temperature can be determined by differential scanning calorimetry (DSC). 2 mg of the sample in the aluminum pans and heated at the 5°C /min, a temperature range of 20°C to 300°C under nitrogen atmosphere of flow rate 30 ml/min.

d. X-Ray Diffraction Studies [9]

Ziziphus xylopyrus phospolipid complex was evaluated in by powder x-ray diffractometry. The XRD spectra compared with crystallinity. A Philips 1710 X-ray Diffractometer (XRD) with a copper target and nickel filter was used to obtain XRD result for the sample. The XRD pattern of each sample was measured from 10 to 50 degrees.

e. *In* vitro Dissolution Studies [10]

In vitro dissolution studies were performed using USP XXIII Dissolution test apparatus II (basket type). weighed sample of phytosomes was taken into 900 mL of 0.1 N HCl, pH 1.2, maintained at a temperature of 37ºC ± 0.5ºC and stirred at a speed of 50 rpm. At different time intervals, a 5 ml of the sample was withdrawn and sink condition they can maintained. After 2 hr's same procedure was repeated into phosphate buffer 6.8 pH. The collected samples were filtered using Whatman filter paper and analyzed at λ_{max} 271 nm using a UV-Visible spectrophotometer against 0.1N HCl and phosphate buffer 6.8 pH as blank. The result measure with the mean of three values.

f. *In vitro* Radical Scavenging Activity of Phytosomes by DPPH model [10]

DPPH is free radical they can accept an electron to form stable diamagnetic molecule. DPPH is efficient radical trap is strong inhibitor of mediated polymerization.

Procedure

1. To give clean and dry test tube contain methanol to make final volume 3 ml, 50 µl of DPPH reagent was added with micro-syringe and mixed thoroughly.
2. The initial absorbance was measured at 517 nm using UV spectrophotometer.

3. The solutions were mixed, allowed to stand for 4min at room temperature and final absorbance was measured at 517nm.
4. The methanolic solution of ascorbic acid (0.5 mg / ml) was added in the range of 5-25 µl as positive control. The % reduction in absorbance was calculated from the initial and final absorbance. 50% reduction in absorbance they can be measured.

Drug-Excipient Compatibility Studies [13]

The physicochemical similarity among blend and of medication and polymer utilized in the exploration product completed by infrared otherworldly examinations utilizing fourier change infrared spectrophotometer. Graph that could be plotted by using potassium bromide dispersion method. The comparisons of the substance and the drug. Thus the infra-red data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the polymer.

***In-Vivo* Anti-Diabetic Activity**

Selection of animals

Wister albino rats of either sex were purchased from Indian institute of toxicology research, Lucknow. All animal were allowed to adapt new environment for 7 days in Daksh Institute of Pharmaceutical Science, Chhatarpur (M.P.), Animal house at suitable environmental condition and provided them standard food product manufactured by Hindustan liver ltd. Above 150 g rats were selected for experiment.

Experimental Induction of Diabetes

Over 150 g rodents of either sex were chosen for this action. Before enlistment of diabetes gauge and ordinary glucose level of rodents was estimated and worried as 0 Day. After overnight fasting 60mg/kg of streptozotocin (Sigma, St. Louis, Mo, USA) newly broke up in 0.1 N sodium citrate supports pH 4.5 was infused intraperitoneally. All creatures came back to their enclosures and given free access to food and water. Blood glucose level was estimated after seventh day, fourteenth day and 21st day. Just rodents with fasting blood glucose level more prominent then 200mg/Dl were viewed as diabetic and remembered for this investigation. Diabetic rodents were arbitrarily doled out to five each, bunch contains six creatures. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) (Protocol no. DIPS/044/2019).

Experimental Design

Group I- Control rats received vehicle solution normal saline.
Group II- Diabetic control rats received vehicle solution normal saline.
Group III – Diabetic rats treated with methanolic extract of *Ziziphus xylophyrus* 200 mg/kg.
Group IV – Diabetic rats treated with methanolic extract of *Ziziphus xylophyrus* 400 mg/kg.
Group V- Diabetic rats treated with Glibenclamide 0.25 mg/kg of body weight in normal saline.

Formulation of Loaded Drug In Capsules [11]

Table 1: Formulation table of capsule

S. No	Ingredient name	Concentration (Mg)
1	*Ziziphus xylopyrus* Phytosome	241.241
2	Dicalcium phosphate dehydrate	84.759
3	Microcrystalline cellulose	47.0
`4	Croscarmellose sodium	23.0
5	Talc	2.0
6	Magnesium stearate	2.0

By using these ingredients granules were prepared by dry granulation method and capsules were filled manually.

Evaluation of drug loaded in capsule form

1. Uniformity of weight [14]

Accurate weighed of 20 capsule average weighed was determine then % deviation calculate. As per IP limit average wt not more than 300 mg it show 7.5% deviation and less than 300mg it show 10% deviation.

2. Content uniformity [14]

30 capsules are given, 10 of which were assayed. The requirement met if 9 of 10 are within specified potency range of 85 to 115% and tenth is not outside 75 to 125%. If more than 1, but less than 3 of first 10 capsules fall outside the 85 to 115% limits, the reaming 20 are assayed. The requirements are met if all 30 capsules are within 75 to125% of the specified potency range, and not less than 27 of the 30 are within the 85 to 115% range.

Statistical Evaluation

The data were statically analyzed by one way ANNOVA followed by dunnet's t-test and values were considered significant. And value were expressed + SEM. & $p<0.05$.

Result and Discussion

UV Spectroscopic Method for the Estimation of *Ziziphus xylopyrus* Leaves Extract

Calibration curve of *Ziziphus xylopyrus* leave extract was determine with 20,40,60,80,100 ppm concentration they can prepared, the wavelength selected for 271 nm, the absorbance value were plotted against concentration to obtain the standard calibration curve.

Table 2: Calibration Curve of Leave Extract.

Conc (µg/mL)	Absorbance at 271nm.
20	0.162
40	0.261
60	0.431
80	0.587
100	0.686

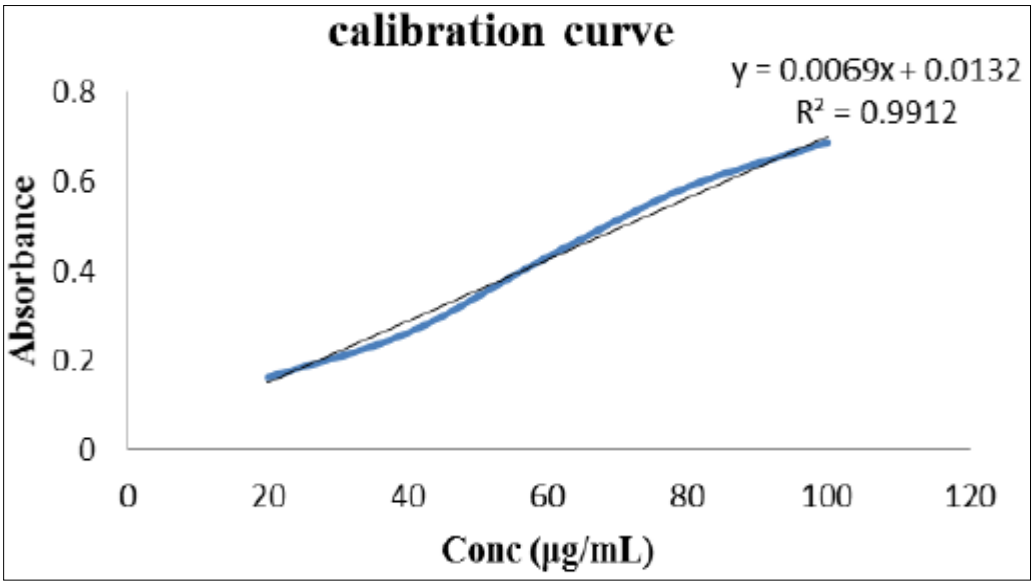

Fig 1: Calibration Curve of *Ziziphus xylopyrus* Leaves

Preparation of Phytosomes

Formulation of phytosome they can prepare in three different ratios 1:0.8, 1:1, 1:1.2. Standard ratio selected for 1:1 ratio given in literature. The entrapment efficiency are given in following table-

Table 3: Entrapment Efficiency of Various Formulations.

S. No.	Formulation No.	Ratio of extract and soya lecithin	Entrapment efficiency(w/w)
1	F1	1:0.8	90.72
2	F2	1:1	94.55
3	F3	1:1.2	94.88

Ratio 1:1.2 was best result they can selected for final formulation

Evaluation of Phytosome Extract

a. Solubility Study [15]

The solubility they can performed in *Ziziphus xylopyrus* methanolic fraction, phospholipid complex and lipid physical mixture in solvent like chloroform by following table-

Table 4: Solubility Study of Phytosomes in Chloroform

Time (hr)	UV abs at 271 nm	Slope	Intercept	µg/ml	mg/ml
Fraction	0.166	0.006	0. 013	25.5	0.0255
Complex	0.54	0.006	0.013	87.83333	0.087
Physical mixture	0.17	0.006	0.013	26.16667	0.026

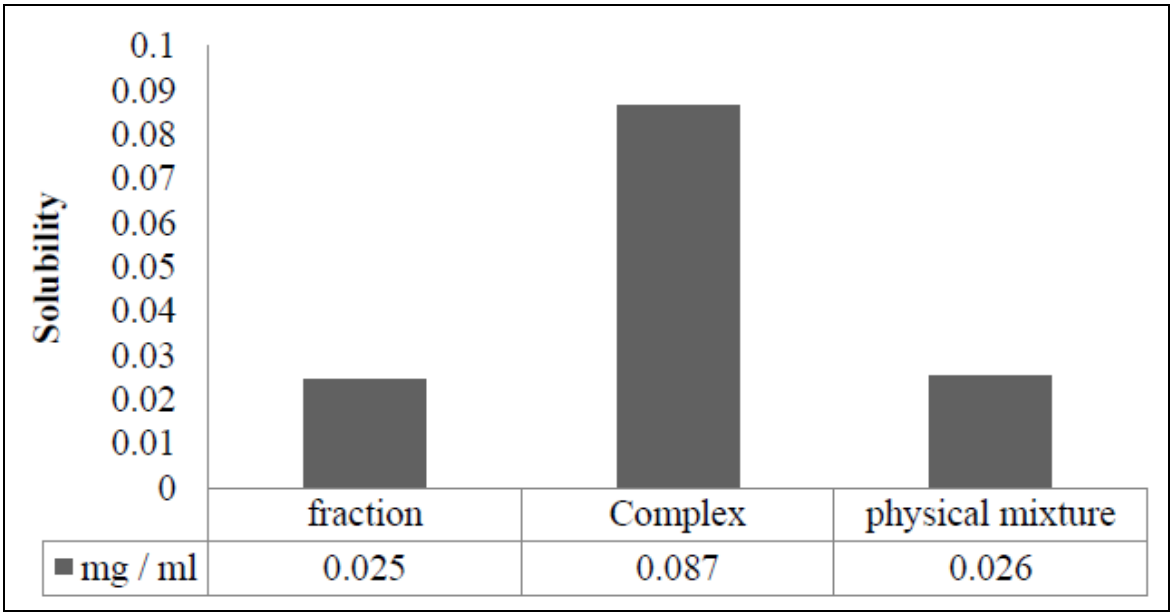

Fig 2: Solubility Study of Phytosomes in Chloroform

b. Differential Scanning Calorimetry

DSC thermogram were obtained to describe the physical state of drug and polymer and also to detect the interaction between drug and polymer in capsule formation. The peak at 204.16°C could be due to hot melt movement of polar head group of soya lecithin. The second peak at 239.89°C might be as a result of phase transition from gel to liquid crystalline.

Table 5: DSC Data of soya lecithin, *Ziziphus xylopyrus* extract and complex

Sample	Onset °C	No of peaks	Major Peaks °C
Soya lecithin	193.55	1	206.66
Ziziphus xylopyrus extract	210.93	2	215.09, 276.75
Complex	199.41	3	204.16, 239.89, 263.00

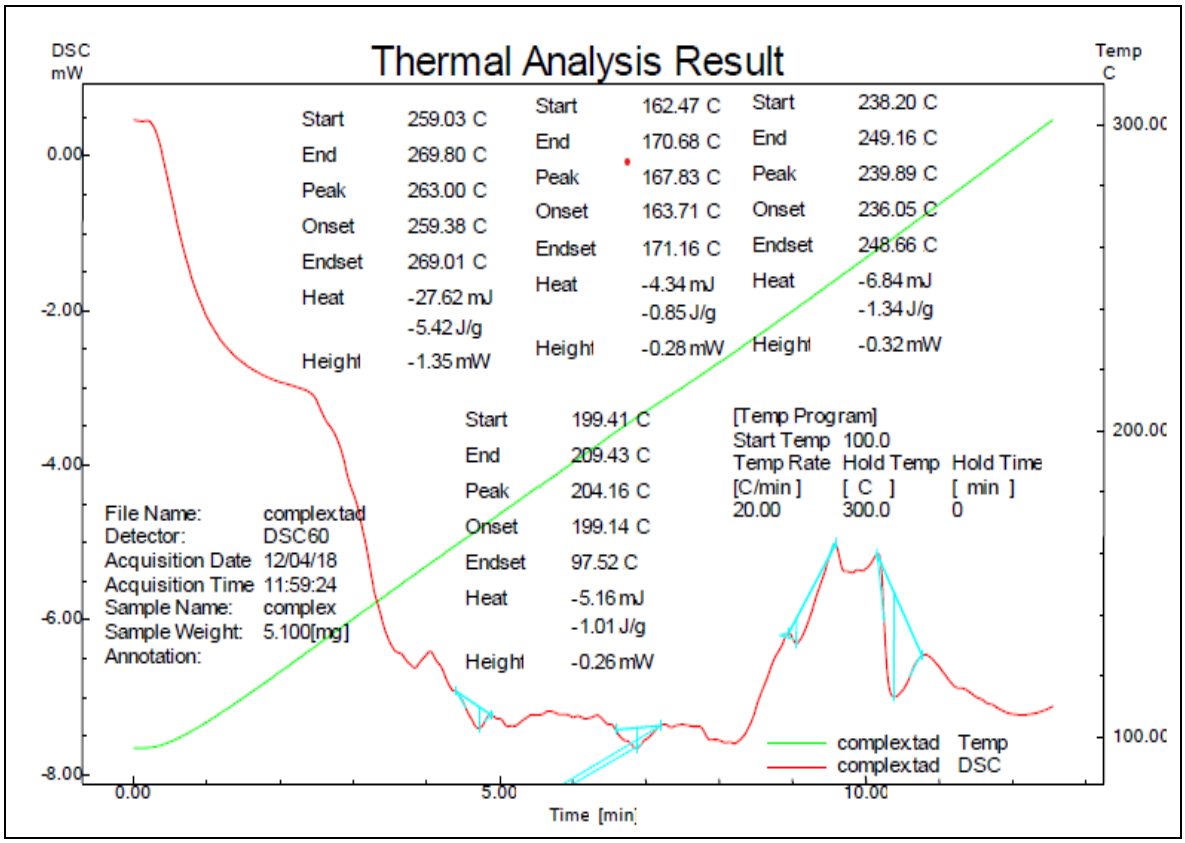

Fig 3: DSC of Complex

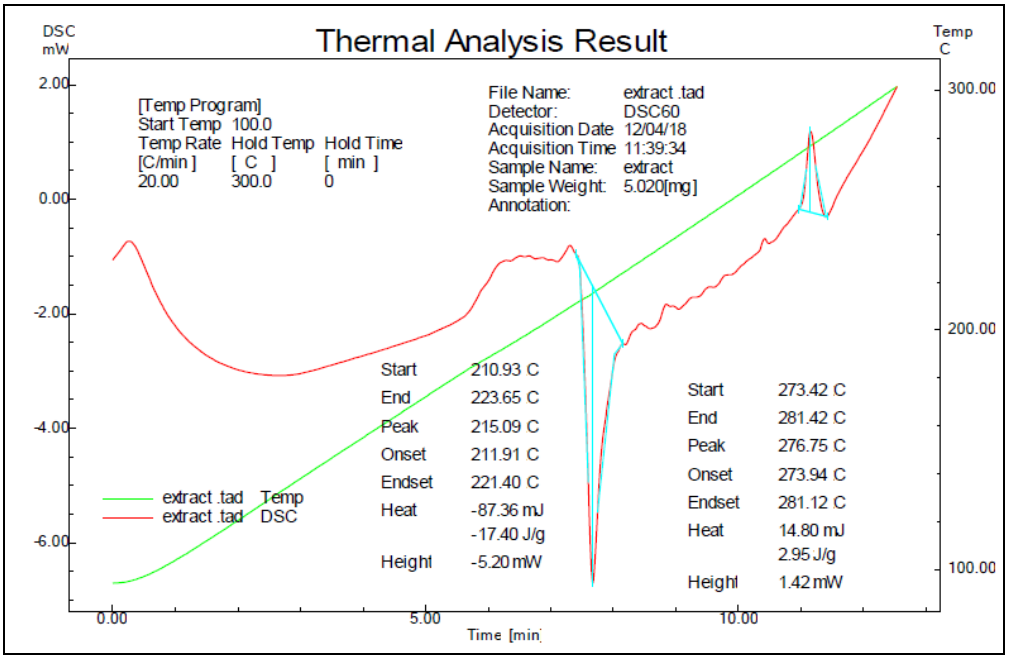

Fig 4: Differential Scanning Calorimetery of extract of *Ziziphus xylopyrus*

c. X-ray diffraction of formulation [16]

The crystalline pattern of drug they can determine by x-ray diffraction technique. The crystalline nature of the drug was demonstrated by the characteristic XRD pattern with peaks appearing at 14.5, 14.9, 15.7, 16.8, 17.8, 18.6, 20.6, 22.1, 22.8, 26.3, 26.8, 29.2, 32.2 and 33.1 θ value.

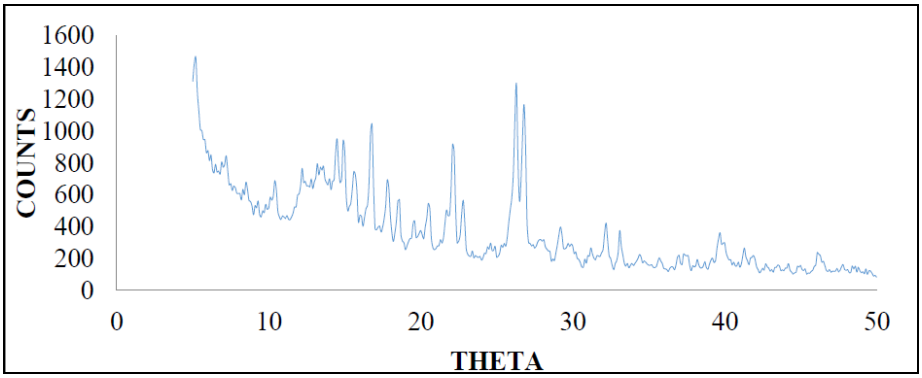

Fig 5: X-ray Diffractogram of *Ziziphus xylopyrus* Extract.

d. *In Vitro* Dissolution Studies of Phytosomes [18]

Absorbance of phytosome in phosphate buffer pH 6.8 was prepared with conc in 0, 2, 4, 6, 8, 10, 12 ppm at 271 nm wavelength. The regression coefficient was found.

In vitro dissolution study indicated that the phytosomes had extended release dissolution patterrn. The phytosomes show of 6 hr. 80.36 % release.

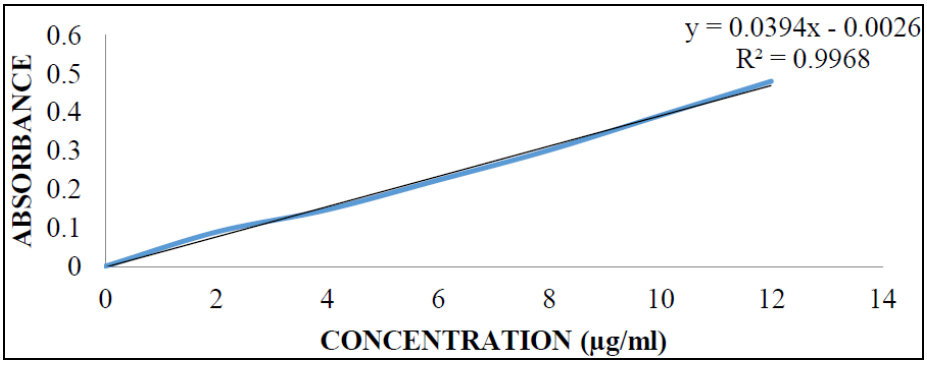

Fig 6: Standard graph of phytosomes in phosphate buffer pH 6.8

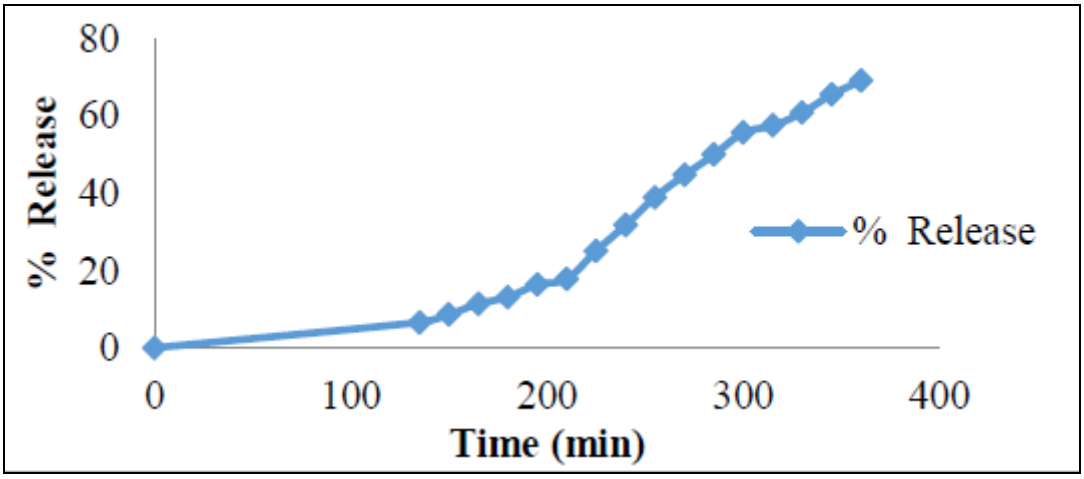

Fig 7: Graph of *in vitro* dissolution study in 6.8 pH Phosphate Buffer of Phytosmes.

Table 6: *In vitro* Dissolution Study in 6.8 P^H Phosphate Buffers

Time	Abs	Slope	Intercept	µg/ml	µg/ml *10	µg/900ml	mg/ml %	Release
0	0	0.039	0.002	0	0	0	0	0
135	0.071	0.039	0.002	1.769231	17.69231	15923.08	15.92308	6.600485
150	0.092	0.039	0.002	2.307692	23.07692	20769.23	20.76923	8.609329
165	0.121	0.039	0.002	3.051282	30.51282	27461.54	27.46154	11.38345
180	0.139	0.039	0.002	3.512821	35.12821	31615.38	31.61538	13.10531
195	0.173	0.039	0.002	4.384615	43.84615	39461.54	39.46154	16.35772
210	0.188	0.039	0.002	4.769231	47.69231	42923.08	42.92308	17.79261
225	0.264	0.039	0.002	6.717949	67.17949	60461.54	60.46154	25.06271
240	0.334	0.039	0.002	8.512821	85.12821	76615.38	76.61538	31.75886
255	0.409	0.039	0.002	10.4359	104.359	93923.08	93.92308	38.9333
270	0.469	0.039	0.002	11.97436	119.7436	107769.2	107.7692	44.67285
285	0.524	0.039	0.002	13.38462	133.8462	120461.5	120.4615	49.93411
300	0.584	0.039	0.002	14.92308	149.2308	134307.7	134.3077	55.67366
315	0.603	0.039	0.002	15.41026	154.1026	138692.3	138.6923	57.49118
330	0.638	0.039	0.002	16.30769	163.0769	146769.2	146.7692	60.83926
345	0.687	0.039	0.002	17.5641	175.641	158076.9	158.0769	65.52656
360	0.724	0.039	0.002	18.51282	185.1282	166615.4	166.6154	69.06595

e. Fourier Transform Infrared (FTIR) Study To Check Stability

The IR peaks were matching which indicated that drug remained same in the complex and did not change the structure.

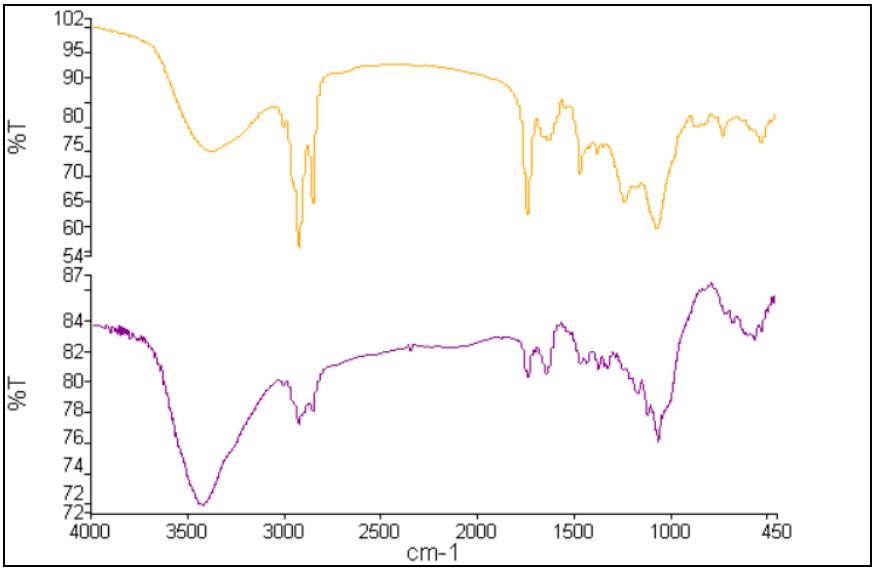

Fig 8: Split FTIR Spectra of ZX Extract and Complex

f. *In* Vitro Radical Scavenging Activity of Phytosomes By DPPH

Table 7: *In Vitro* Free Radical Scavenging Activity of Phytosomes by DPPH

Conc	Abs. of DPPH	abs of test	Inhibition	% inhibition
5.00	0.648	0.402	0.37963	37.96296
10.00	0.648	0.358	0.447531	44.75309
20.00	0.648	0.339	0.476852	47.68519
30.00	0.648	0.254	0.608025	60.80247
40.00	0.648	0.297	0.541667	54.16667
50.00	0.648	0.193	0.70216	70.21605
60.00	0.648	0.153	0.763889	76.38889
70.00	0.648	0.127	0.804012	80.40123
80.00	0.648	0.087	0.865741	86.57407
90.00	0.648	0.057	0.912037	91.2037
100.00	0.648	0.018	0.972222	97.22222

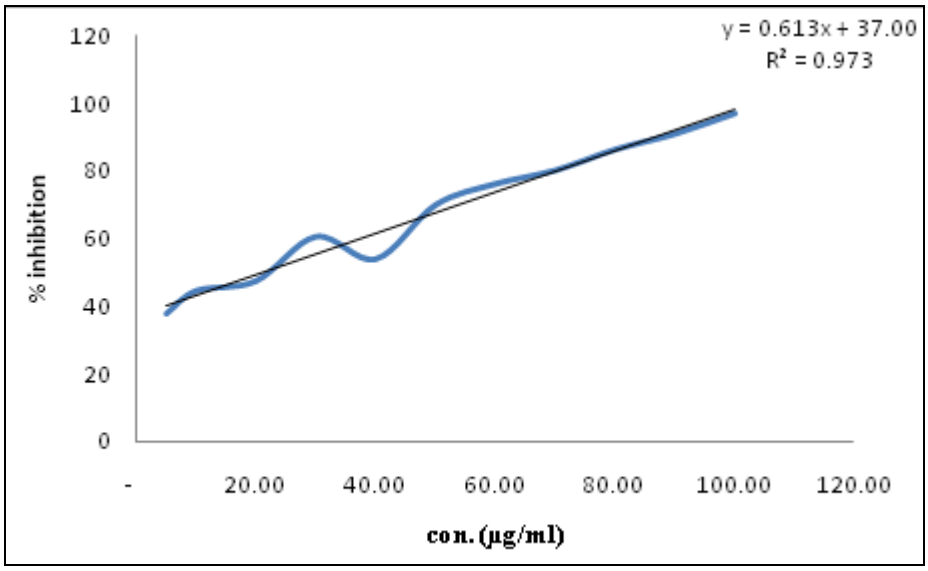

Fig 9: *In vitro* free Radical Scavenging Activity of Phytosomes by DPPH

1. Evaluation of Loaded Drug in Capsules

1) *In Vitro* Dissolution Test for Phytosomes Dosage Form Capsules [21, 23, 24]

Table 8: *In Vitro* Drug release of Phytosomes Capsule in 6.8 P^H Phosphate Buffer

Time	Abs	Slope	Intercept	µg/ml	µg/ml *10	µg/900ml	mg/ml %	Release
0	0	0.039	0.002	0	0	0	0	0
135	0.068	0.039	0.002	1.692308	16.92308	15230.77	15.23077	6.313508
150	0.083	0.039	0.002	2.076923	20.76923	18692.31	18.69231	7.748396
165	0.128	0.039	0.002	3.230769	32.30769	29076.92	29.07692	12.05306
180	0.146	0.039	0.002	3.692308	36.92308	33230.77	33.23077	13.77493
195	0.167	0.039	0.002	4.230769	42.30769	38076.92	38.07692	15.78377
210	0.192	0.039	0.002	4.871795	48.71795	43846.15	43.84615	18.17525
225	0.271	0.039	0.002	6.897436	68.97436	62076.92	62.07692	25.73233
240	0.345	0.039	0.002	8.794872	87.94872	79153.85	79.15385	32.81111
255	0.407	0.039	0.002	10.38462	103.8462	93461.54	93.46154	38.74198
270	0.485	0.039	0.002	12.38462	123.8462	111461.5	111.4615	46.2034
285	0.527	0.039	0.002	13.46154	134.6154	121153.8	121.1538	50.22108
300	0.593	0.039	0.002	15.15385	151.5385	136384.6	136.3846	56.53459
315	0.624	0.039	0.002	15.94872	159.4872	143538.5	143.5385	59.50003
330	0.671	0.039	0.002	17.15385	171.5385	154384.6	154.3846	63.99601
345	0.713	0.039	0.002	18.23077	182.3077	164076.9	164.0769	68.0137
360	0.754	0.039	0.002	19.28205	192.8205	173538.5	173.5385	71.93572

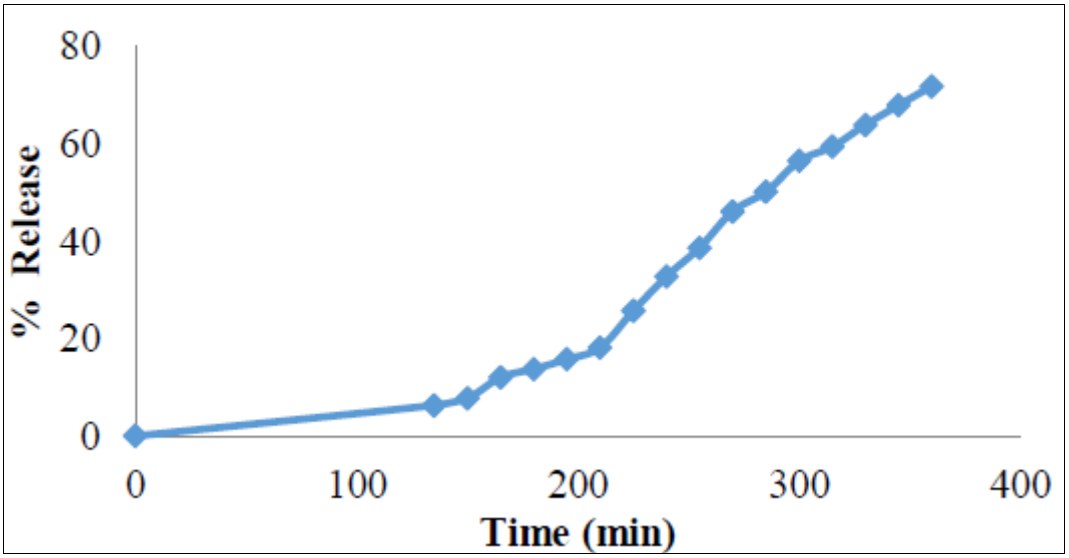

Fig 10: *In Vitro* Drug release of phytosomes capsule in 6.8 pH phosphate buffer.

In vitro drug release of phytosomes formulation, capsule showed at the end of 6 hr 83.51 % release. The only phytosomes showed at the end of 6 hr. 80.36 % release. Both results indicated that, there was not much effect of excipient on phytosomes capsules.

Uniformity of weight

The % deviation was found to be 5.5 %, so it passes uniformity of weight test as per IP.

Content Uniformity

A fundamental quality attribute for all pharmaceutical preparations is the requirement for a constant dose of drug between individual capsules. Uniform drug content was observed for the entire capsules (93.5-105.83 %) % Drug Content.

Table 9: Stability of Drug Content

Sampling Interval	% Drug content
	40°C/ 75% RH
0th Day	97.33
7th Day	96.34
15th Day	95.33
21st Day	94.16
30th Day	93.50

Stability Studies

Stability studies were carried out on phytosomes containing capsules, according to ICH guidelines. The stability studies were carried out at 40ºC/75% RH for 30 days. The samples were tested for drug content after 0, 7, 15, 21 and 30 days. In vitro drug release of the capsule 83.51%, uniform drug content was observed for all capsule (93.5-105.83%).The prepared formulation was stable for storage period.

Table 10: Anti-diabetic Effect

Treatment & Dose	Serum glucose level mg/dl time after treatment (days)			
	0	7	14	21
Group-I Normal Control	90±4.8	95±3.5	93±1.8	97±2
Group-II Diabetic Control	500±4	505±3.0	500±4	503±3.5
Group-III *Ziziphus xylopyrus* Extract 200mg/kg bw	503±3.6	163±3.9*	128±10.7*	125±5.4*
Group-IV *Ziziphus xylopyrus* Extract 400mg/kg bw	511±4.2	262±4.5*	205±2.8*	208±2.3*
Group-V Standard Drug Glibenclaminde 0.25mg/kg bw	510±3.9	388±3.9*	345±6.0*	348±6.0*

Conclusion

The leaves of *Ziziphus xylopyrus* extract having liver protecting activity. shinoda and lead acetate test was found to be positive for both aqueous extract of *Ziziphus xylopyrus* and methanolic fraction. Phytosome study they can increases therapeutic efficacy, decrease the frequency of administration. Phytosome formulated with solvent evaporation method. The different formulation prepare 1:0.8, 1:1, 1:1.2 ratio. The best formulation selected

ratio1:2 for final formulation. *In vitro* dissolution study of phytosome extended release pattern show 6hr, 80.36 releases. The synergistic effect determined by free radical scavenging activity of *Ziziphus xylopyrus* phytosome using DPPH model. In conclusion, phytosome successfully prepared and encapsulated.it show extended release pattern with enhanced free radical scavenging activity. *Ziziphus xylophyrus* drug shows hepatoprotective activity as well as they are traditionally used in the treatment of diabetes.

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