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FORMULATION AND EVALUATION OF PHYTOSOMES CONTAINING BIOACTIVE FROM CARICA PAPAYA SEEDS

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ABSTRACT

Background: Papaya seeds are a rich source of proteins, fat, fibers, vitamins, minerals, monounsaturated fatty acids, polyphenols, and powerful antioxidants like flavonoids. Low solubility limits the absorption and bioavailability of herbal constituents. Hence, phytosomes of Papaya seed extract were formulated to enhance its solubility and bioavailability. **Methodology:** Papaya seeds were extracted using ethanol as solvent, and all the phytoconstituents present in the extract were assessed during the phytochemical screening and LC-MS analysis. In-vitro antidiabetic activity pure extract was determined by alpha-amylase and alpha-glucosidase enzyme inhibitory assay. The phytosomes of extract were formulated using the lipid thin film formation method and Soya lecithin and Cholesterol as lipids. The formulated phytosomes were analyzed for parameters such as particle size, zeta potential, encapsulation efficiency, percent drug content, and In-vitro dissolution study. The chemical nature of the formulation was studied using FTIR analysis and powder X-ray diffractometry. Thermal stability of phytosomes analyzed with the help of Differential Scanning calorimetry. **Results:** LC-MS identified 16 phytoconstituents. In-vitro antidiabetic activity showed 59.97% and 51.17% inhibition of enzymes alpha-amylase and alpha-glucosidase, respectively. The encapsulation efficiency of the optimized formulation was 88.41±0.91% with a particle size of 188.0±53.7nm. TEM images of formulation confirm the formation of phytosomes. FTIR, DSC, and Powder X-ray diffractometry showed no unwanted peaks. The in vitro dissolution study showed 89.26±1.05% CDR of phytosome, while the extract showed 47.78±0.59% CDR. **Conclusion:** Evaluation results of phytosomes suggest that this formulation can be used as an effective herbal antidiabetic formulation.

INTRODUCTION

Phytosomes are tiny particles or cells that enhance herbal products' absorption & pharmacokinetic and pharmacodynamic profiles compared to traditional extracts. Phytosome is the term derived from two words, Phyto and some, in which phyto indicates plant and some indicates cell-like [1]. Phytosomes are

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a vesicular drug delivery system that increases the absorption rate of poorly soluble drugs [2]. Phytosome structures bind the active ingredients of standardized plant extracts to phospholipids, primarily phosphatidylcholine, forming a lipid-compatible complex [3]. Phytosomes are formed by reacting a measured quantity of phospholipid with standardized extracts or phenolic components (such as flavonoids) in non-polar solvents [4]. Phytosomes are advantageous over conventional herbal preparations in the following ways [5]:

- Enhanced absorption of lipid insoluble botanical extracts through oral and topical route
- Dose is minimized
- Better drug entrapment
- Nutritional value of excipients used in formulation (Phosphatidylcholine)
- Good chemical stability due to formation of chemical bonds
- Systemic targeting of herbal drugs

The *Carica papaya* belongs to the *Caricaceae* family, which includes various species traditionally used as remedies for several types of diseases [6]. Although papaya fruit is a rich source of phytoconstituents, Papaya seeds contain numerous phytochemicals, including flavonoids, phytosterols, carotenoids, alkaloids, phenolic compounds, and cyanogenic compounds (benzyl glucosinolate), that give the seeds various therapeutic effects [7,8]. Seeds account for approximately 20% of the weight of papaya fruit. Despite being medicinally useful, Papaya seeds usually get wasted when the fruit is being used [9]. *Carica papaya* seeds possess various pharmaceutical effects like antifertility, anti-inflammatory, anthelmintic, contraceptive analgesic antibacterial quality, carminative, emmenagogue, abortifacient, and counterirritant [10,11]. *Carica Papaya* seeds can further manage hypertension and hypercholesterolemia [12,13].

Antidiabetic and hypolipidemic effects of Carica Papaya seeds have been reported in several studies [7,14,15]. The inhibition of α-glucosidase and α-amylase enzymes in type 2 diabetes may be responsible for the hypoglycemic effect of seeds [16]. Phytoconstituents of papaya seeds, such as flavonoids, quercetin, steroids, phenols, quinones, and kaempferol, can significantly lower blood sugar levels by recovering beta cells of the pancreas and increasing insulin secretion [17]. As the important phytoconstituents of papaya seed, such as flavonoids and phenols, have poor solubility, the present study aimed to formulate and evaluate phytosomes containing bioactive from *Carica papaya* seeds to improve the solubility and bioavailability of papaya seed extract for effective use against diabetes.

MATERIALS AND METHODS

Chemicals

Petroleum ether, Ethanol, Sodium Hydroxide, Copper Sulphate, Nitric Acid, Millon's Reagent, Benedict's Reagent, α- Naphthol, Fehling's Solution A and Fehling's Solution B, Wagner's reagent, Mayer's Reagent, Ferric Chloride, Acetic anhydride, Conc. Sulphuric acid, Chloroform, Acetic acid, Glacial acetic acid, α- Amylase, Soluble starch, DNS color reagent, Acarbose, α-Glucosidase, Cholesterol, Soya lecithin, Methanol, Phosphate buffer.

Plant materials

Ripe *Carica papaya* fruits were collected, and a botanist performed the authentication of the plant, Dr. Hemantkumar A. Thakur, at the Department of Botany, Gokhale Education Society's H.P.T. Arts & R.Y.K. Science College, Nashik. The seeds were collected and washed using water, after which they dried under shade for 15 days. The dried seeds were then ground into a powder in a lab mixer so that they could be extracted.

Plant Extraction:

100 g of *Carica papaya* seed powder was first extracted using a Soxhlet Apparatus with petroleum ether to remove fats and then extracted with ethanol (95%). The Soxhlet extraction method was used for the extraction of papaya seeds, as it efficiently extracts compounds, shortens extraction time, and gives higher yields as compared with traditional extraction methods [18]. The extract was evaporated at a temperature of not more than 60°C. The residue was collected and stored at 4°C.

Phytochemical Testing:

In phytochemical screening, the presence of proteins, saponins, carbohydrates, alkaloids, phenols, tannins, flavonoids, steroids, and glycosides was examined [19].

LC-MS analysis:

LC-MS of Papaya extract was carried out using Agilent 6550 Q-TOF LC-MS instrument. Targeted LC-MS screening of the extract was performed to identify the phytoconstituents present in the crude extract. The run time for screening was 60 min on negative mode.

In vitro determination of % Inhibition of enzyme alpha-amylase [20]:

In a 96-well plate, the mixture having 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α–amylase (2 U/ml), and 20 µl of different amounts of extract and standard (1,2,4,8,12,16,20 mg/ml) was first incubated at 37°C for 20 min. Then, the 20 µl of 1% starch (100mM phosphate buffer pH 6.8) was used as a precursor, incubation was carried out at 37°C for 30 min; 100 µl of the DNS color reagent was mixed and allowed to boil for 10 min. The absorbance of the final preparation was then determined at 492 nm using a Multiplate Reader. Acarbose was compared as a standard with different concentrations (1.0–20 mg/ml). A control (no extract) substance was also measured simultaneously, and every experiment was repeated in duplicates. The findings were represented as percent inhibition, which was determined with the use of the given formula:

% Inhibition = (Ac-At/Ac) ×100

Where, Ac = Absorbance of Control, At = Absorbance of Test

In vitro determination of % Inhibition alpha-glucosidase [20]:

In a 96-well plate, a preparation of 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl extract and standard concentrations (1,2,4,8,12,16,20 mg/ml) was first incubated for 15 minutes at 37°C. The substrate, 20 µl P-NPG (5 mM), was incorporated and incubated for 20 minutes at 37°C. 50 µl of 0.1 M NaCl was incorporated to finish the reaction. The p-nitrophenol formed was measured by measuring the absorbance at 405 nm using a Multiplate Reader. Acarbose was compared as a standard with different concentrations (1.0–20 mg/ml). A control (no extract) substance was also measured simultaneously, and every experiment was repeated in duplicates. The findings were represented as percent inhibition, which was determined with the use of the given formula:

% Inhibition = (Ac-At/Ac) ×100

Where, Ac = Absorbance of Control, At = Absorbance of Test

Formulation of phytosomes loaded with Carica papaya seed extract:

The phytosomes were formulated using the thin film hydration method. Shortly, Papaya extract, soy lecithin, and cholesterol were solubilized in 100 ml of chloroform, which was then evaporated in a rotary flash evaporator at 75 rpm at 45°C to form a film of lipid and extract. In this film, then phosphate buffer saline (pH 7.4) was added for hydration and kept it for 1 hr. This mixture was then sonicated in an ice bath for 30 min. The phytosomes were given stability by hydrating them for 8 hours. The formed phytosomes were centrifuged to separate them from the free drug. The supernatant was discarded, and the prepared phytosomes were freeze-dried [21,22].

Optimization of formulation parameters:

The formulation method was optimized by analyzing process-related variables like concentration of cholesterol and lipid [21,22]. The cholesterol-lecithin ratio was an independent variable (X1 and X2), resulting in a 3^2 factorial design with nine possible combinations. The dependent variables were defined as encapsulation efficiency and in-vitro drug release. Design Expert 13 software was utilized to investigate the impact of independent variables on dependent variables. Table 1 represents the composition of phytosomes and their formulation codes.

Table 1: Composition of phytosomes containing different concentrations of Cholesterol and lecithin

Code	Extract (mg)	Cholesterol (mg) (X1)	Soya lecithin (mg) (X2)	Cholesterol lecithin ratio
F1	100	50	100	0.5:1
F2	100	50	200	0.5:2
F3	100	50	300	0.5:3
F4	100	100	100	1:1
F5	100	100	200	1:2
F6	100	100	300	1:3
F7	100	150	100	1.5:1
F8	100	150	200	1.5:2
F9	100	150	300	1.5:3

Evaluation of Phytosmes:

Encapsulation Efficiency (EE) and Drug content determination:

The encapsulation efficiency of the phytosome was evaluated with the help of a UV spectrophotometer. A weighed amount of phytosomes (10mg) was solubilized in 10 ml methanol. The mixture was sonicated for 15 minutes to ensure the complete dissolution of phytosomes and the release of the entrapped drug. The mixture was then further diluted, and the entrapped bioactive in phytosomes were measured at 273nm. The encapsulation efficiency was calculated by using the given formula [23]

$$EE = \frac{amount\ of\ entrapped\ drug}{Amount\ of\ initial\ drug} \times 100$$

The drug content was determined using the following formula:

$$Drug\ content = \frac{amount\ of\ drug\ in\ complex}{Amount\ of\ complex} \times 100$$

Particle size, polydispersity index, and zeta potential determination:

Particle size, polydispersity index, and zeta potential of phytosomes were found by the Horiba Scientific SZ-100-Z2 particle size analyzer. It measures particles' size and distribution width by dynamic light scattering (DLS). 5mg of sample was dispersed in 10 ml deionized water for particle size determination. Further dilutions were performed whenever required.

Transmission Electron Microscopy (TEM):

TEM is an analytical technique used to visualize the smallest particles. The surface morphology of prepared phytosomes was studied using TEM (Model: ECNAI 12, Netherlands, Software: Tecnai imaging & Analysis; Source – Tungsten Filament). An electron beam is passed through the thin sample to form a picture. This technique gives the substance's complete physical, crystallographic, atomical, microanalytical, electronic structure, and coordination number. TEM had an operating voltage range of 20 to 120kV. It magnifies the image up to 700000 times.

Fourier Transform Infra-red spectrophotometry (FTIR)

The FT-IR spectrum of Pure extract, Soya lecithin, Cholesterol, Drug- Excipient mixture, and phytosome were recorded using Bruker FT-IR Alpha II Fourier Transform Infrared Spectrophotometer. A small sample quantity (5-10 mg) was directly placed on a diamond crystal of IR, the pressure arm was adjusted over the sample, and pressure was applied to the sample. The spectra were recorded by using the software. The spectra were observed for any interactions between extract and excipients in the physical mixture for drug-excipient compatibility study. Any physicochemical interaction was observed in the formulation.

Differential Scanning Calorimetry (DSC)

Heat stability of formed phytosome was accessed by differential scanning calorimetry model no. DSC 4000 by Perkin Elmer. It uses several quick heating and isothermal hold steps to cover the interest's temperature range. 9 mg of sample was sealed in an aluminum pan, and a DSC thermogram was recorded at a heating rate of 10°C/min from temperature range of 30°C to 400°C

Powder X-ray diffractometry

The samples of pure extract, Soya lecithin, Cholesterol, and Phytosomes were analyzed to study their physical structure by obtaining X-ray diffraction scans. The scans were performed using X-ray diffractometer (Model no: Smartlab Cu 1.5 KV by Rigaku, Japan). This method employs the PhotonMax high-flux 9 kW rotating anode X-ray source paired with a HyPix-3000 high-energy-resolution 2D multidimensional semiconductor detection system, which supports the 0D, 1D, and 2D measurement modes.

In vitro dissolution studies

The in-vitro dissolution of phytosomes was conducted in the USP Type I dissolution test apparatus (LABINDIA DS 8000) at 50 rpm and at 37°C. The measured amount of phytosomes (100 mg) was filled in size 0 capsule and the capsule is placed in basket which is then put in to 900 ml of phosphate buffer (pH 6.8). Samples (5 ml each) of dissolution fluid were removed at intervals of 1 hour for 12 hours and substituted with the equal amount of fresh medium to keep the sink conditions stable. Withdrawn samples were filtered and evaluated at 273 nm by UV spectroscopy to determine drug release from the phytosomes [24,25].

RESULTS AND DISCUSSION

Phytochemical testing of seed extract

Phenols and flavonoids are the compounds which are found in edible and non-edible parts of the plant which has numerous biochemical activities, including antioxidant, antidiabetic, anti-inflammatory, antimutagenic, and anticarcinogenic properties [26,27]. *Carica papaya* L. (Caricaceae), also known as pawpaw, is a popular and cost-effective fruit tree with high nutritional value [28]. Table 2 summarizes the presence of phytochemical constituents found in ethanolic *Carica papaya* seed extract.

LC-MS Spectra

LC-MS analysis of Extract shows presence of important phytoconstituents like phenols and flavonoids which exhibit in vitro antidiabetic activity. The LC-MS chromatogram of Papaya seed extract is given in figure 1. Targeted LC-MS screening identified 16 bioactive compounds namely Mallic acid, Gallic acid, Protocatechuic acid, Caffeic acid, Clitorin, Rutin, Morin, Luteolin, Morin, Ferulic acid, Isorhamentin, 5,7 Dimethoxycoumarin, p-Coumaric acid, Myricetin, Apigenin, Nicotiflorin.

Table 2: Phytochemical tests of ethanolic extract of *Carica papaya*

Test	Constituent	Result
Biuret test	Proteins	+
Molisch's Test	Carbohydrates	+
Fehling's Test	Carbohydrates	+
Wagner's Test	Alkaloids	+
Mayer's Test	Alkaloids	+
Ferric Chloride test	Phenols	+
Gelatin test	Tannins	-
Braymer's test	Tannins	-
Alkaline Reagent Test	Flavonoids	+
Liberman Burchard test	Steroids	-
Liberman's test	Glycosides	-
Keller Killiani test	Glycosides	-
Foam Test	Saponins	+

In vitro inhibition of enzyme alpha amylase:

The absorbance and percent inhibition of enzyme alpha amylase by papaya seed extract is summarized in table 3. Table 4 shows absorbance and percent inhibitory activity of enzyme alpha glucosidase by extract compared with standard acarbose.

α-amylase and α-glucosidase are enzymes produced by the gastrointestinal tract that digest complex carbohydrates, leading to postprandial hyperglycemia [16]. In vitro enzyme inhibition study showed *Carica papaya* seed extracts had moderate enzyme inhibitory activity when compared to the standard (Acarbose). IC_{50} value (Concentration of extract which causes 50% of inhibition of enzyme) of crude extract was found to be 20 mg/ml. This result showed that crude extract of *Carica papaya* seed has antidiabetic potential.

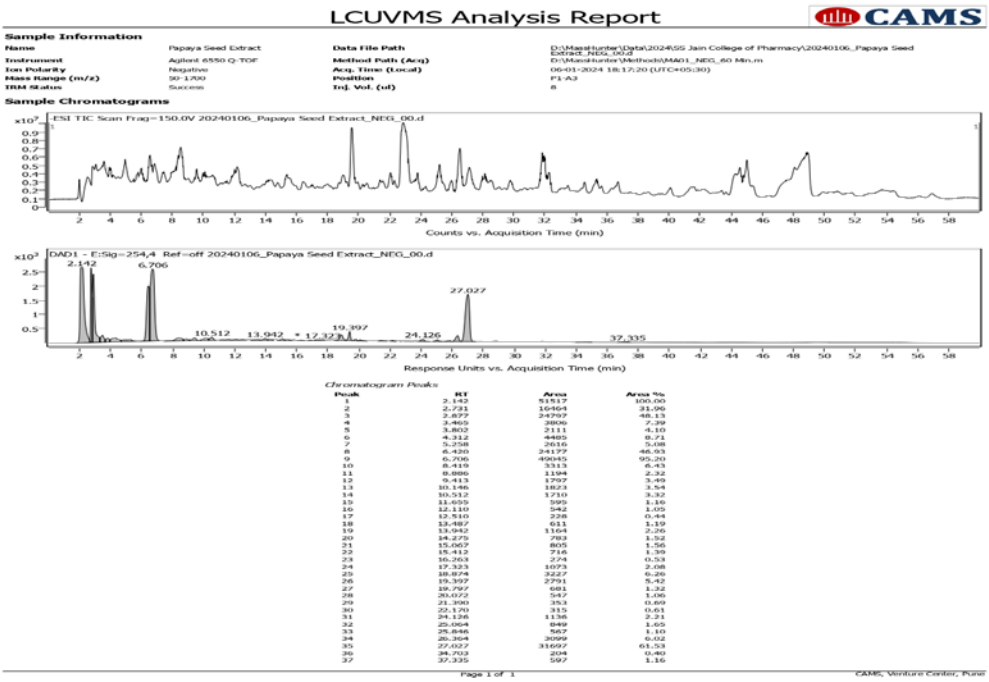


Figure 1: LC-MS graph of Papaya seed extract

Table 3: Absorbance and percent inhibition of enzyme alpha amylase

Absorbance				% Inhibition	
	Extract	Acarbose		Extract	Acarbose
Control	3.96655	3.76145		0	0
1mg	3.4165	2.283		13.86595	44.75668
2mg	3.2625	1.325		17.74968	64.77422
4mg	2.807	0.7505		29.23321	80.04759
8mg	2.281	0.3795		42.49411	89.91081
12mg	2.133	0.2396		46.22531	93.63012
16mg	1.906	0.1799		51.94817	95.21727
20mg	1.5875	0.13865		59.97781	96.31392

Table 4: Absorbance and percent inhibition of enzyme alpha glucosidase

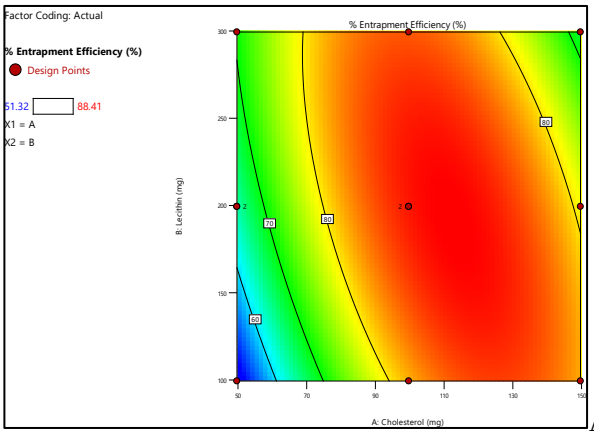
Absorbance				% Inhibition	
	Extract	**Acarbose**		**Extract**	**Acarbose**
Control	2.90975	3.01		0	0
1mg	2.7957	2.6495		3.919581	11.97674
2mg	2.50325	2.0798		13.97027	30.90365
4mg	2.42085	1.868		16.80213	37.9402
8mg	2.0745	1.42775		28.70522	52.56645
12mg	1.8492	0.90655		36.44815	69.88206
16mg	1.5352	0.6605		47.23945	78.05648
20mg	1.4208	0.4187		51.17106	86.0897

Formulation and optimization of phytosomes

Phytosomes were obtained using the lipid film hydration method. The formulation included soya lecithin, which regulates cell membranes, and cholesterol, which gives stability [29]. In the current study, the encapsulation efficiency (%) was obtained in the range of 51.32±1.24 to 88.41±0.91, while the % in-vitro drug release was found in the range of 50.63±0.73 to 89.26±1.04. From the results expressed by Design Expert F5 batch was selected as optimized batch. The results of for Encapsulation efficiency, Drug content and in-vitro drug release for formulation batches are given in table 5. The contour graph and 3D response graph show strong influence of independent variables on dependent variables. The graphs for % EE and % in-vitro drug release are given in figure 2 and 3 respectively. The ANOVA of the responses and model summary statistics for the selected significant models are shown in table 6 and 7 respectively.

Table 5: The values of Encapsulation efficiency, Drug content and in-vitro drug release

Code	Encapsulation efficiency (%EE)	Drug Content (%)	In-vitro drug release (%)
F1	51.32±1.24	12.83±0.31	50.63±0.73
F2	64.17±1.24	16.04±0.31	62.18±0.74
F3	68.76±0.91	17.19±0.23	66.64±0.80
F4	77.99±1.24	19.50±0.31	75.91±0.49
F5	88.41±0.91	22.10±0.23	89.26±1.04
F6	87.92±0.59	21.98±0.15	83.84±1.96
F7	84.53±1.49	21.13±0.37	81.43±0.61
F8	77.50±0.91	19.37±0.31	76.44±0.90
F9	66.84±1.19	16.71±0.30	64.94±0.15



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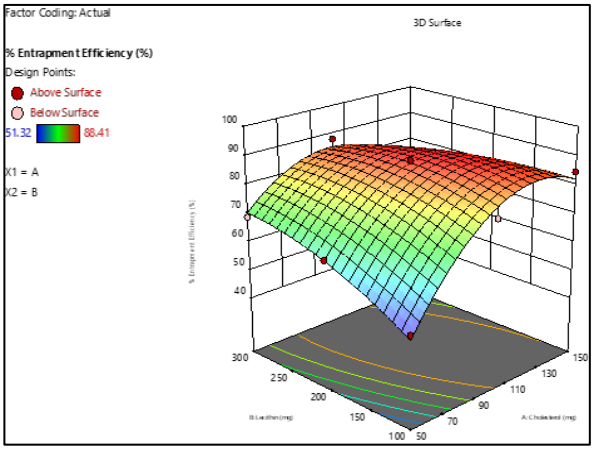
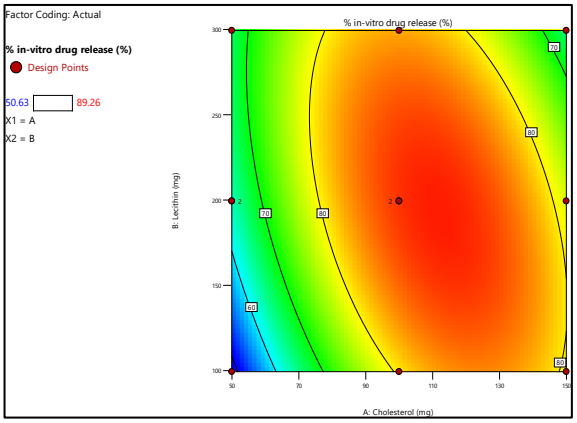


B

Figure 2: The contour plot (A) and 3D response graph (B) as a function of %EE

Table 6: ANOVA for response surface quadratic model for *Carica papaya*

Response	Model F value	P value	Lack of fit	
			F- value	P-value
% EE	38.25	0.0005	46.80	0.0006
%In vitro drug release	34.49	0.0007	46.98	0.0006



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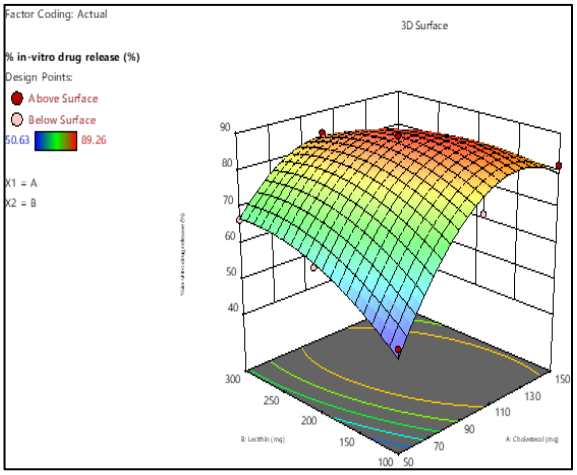

B

Figure 3: The contour plot (A) and 3D response graph (B) as a function of % in vitro drug release

The mathematical representation of prepared phytosomes produced the equations shown below.

%EE (Y1) = 87.71+7.43X1+1.61X2-8.78X1X2-16.15$X1^2$-4.05$X2^2$

%In-vitro drug release (Y2) = 87.40+7.40X1+1.24X2-8.12X1X2-16.76$X1^2$-5.66$X2^2$

Evaluation of phytosomes

After the optimized batch was selected, a fresh formulation of that batch was prepared and evaluated for the following parameters.

Encapsulation Efficiency and Drug content determination:

The encapsulation efficiency of the formulation was found to be 88.41±0.91%. This indicates good entrapment of crude extract in the phytosomes. The drug content of the phytosomes was found to be 22.10±0.226%.

Particle size, polydispersity index and zeta potential determination:

Particle size and zeta potential play important role in physical stability of nano carrier dosage forms in liquid medium. The mean size of particles of formulated phytosomes was 188.0±53.7 nm having polydispersity index of 0.713. The particle size of the particles is below 500 nm which is desired for nano carrier dosage forms with low polydispersity index which indicates narrow distribution of particles. The zeta potential was found to be -53.9mV which indicates excellent stability of phytosomes. Particle size and zeta potential distribution of formulation is shown in figure 4.

Table 7: Model summary statistics-Influence of formulation variables on the response factors for *Carica papaya*.

Response factor	Source	Standard deviation	R^2	Adjusted R^2	Predicted R^2	Adequacy precision
% EE	Quadratic	2.79	0.9745	0.9490	0.7637	18.4305
%In vitro drug release	Quadratic	2.98	0.9718	0.9436	0.7837	17.7957

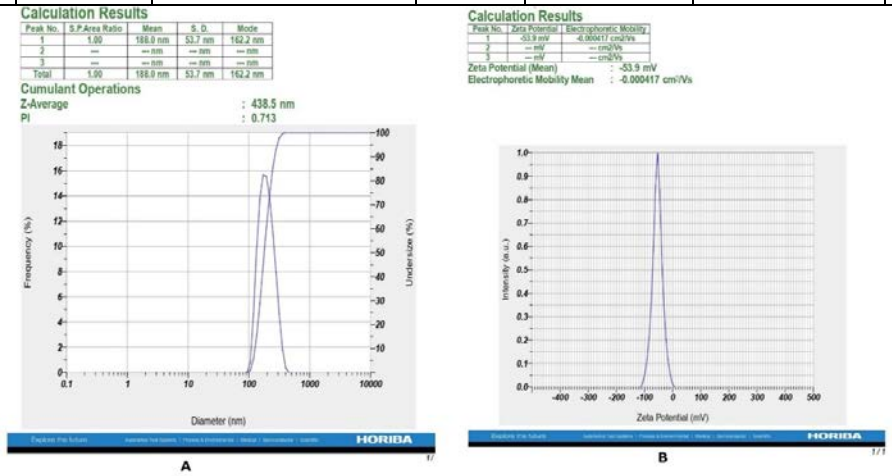

Figure 4: Particle size and zeta potential of phytosome

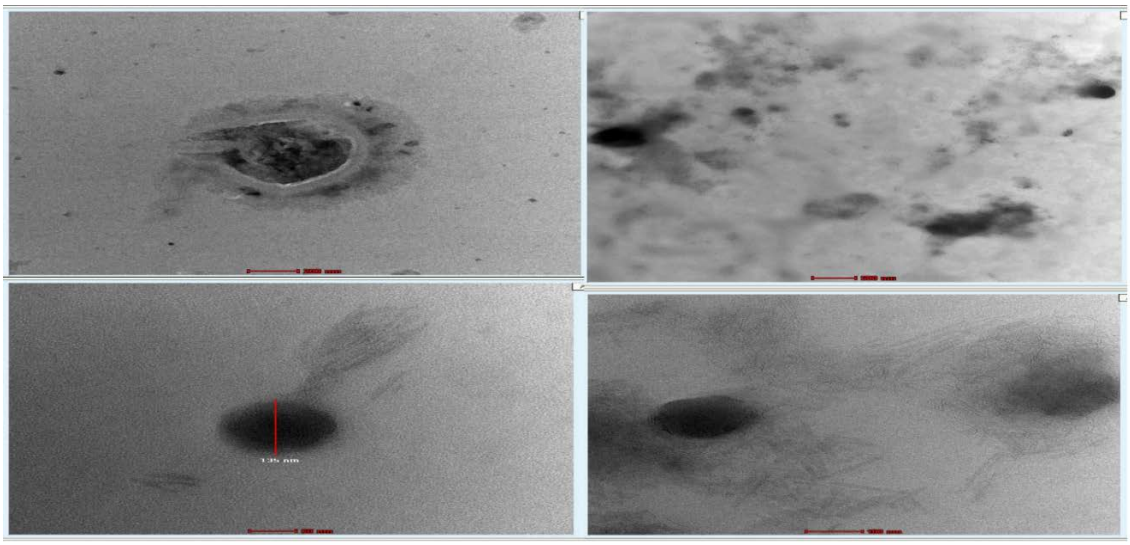

Figure 5: TEM images of Phytosome

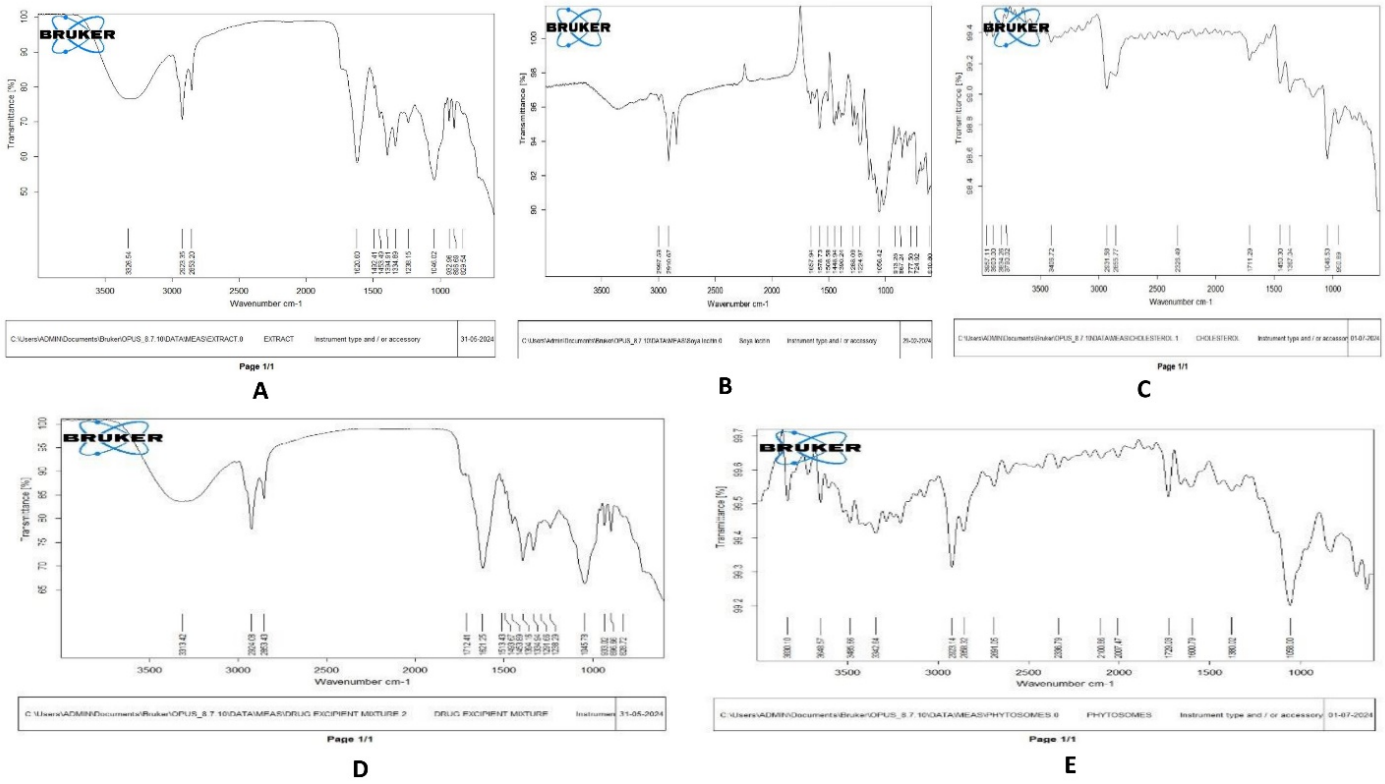

Figure 6: FTIR spectra of (A) Extract, (B) Soya lecithin, (C) Cholesterol, (D) Extract-Excipient mixture, & (E) Phytosome

Transmission Electron Microscopy (TEM)

TEM was used to study the physical structure of phytosomes. The TEM images of the phytosomes are shown in Figure 5. The images show the phytosomes with round shapes and regular size required for phytosomal formulation. TEM surface morphology analysis showed that phytosomes were uniformly spherical and evenly distributed in nano-sized formulations.

Fourier Transform Infra-red spectrophotometry (FTIR)

FTIR spectra of Extract, soya lecithin, cholesterol, Physical mixture, and phytosome are shown in Figure 6. The characteristic FT-IR absorption peaks of extract, 3326.54 (Alcohol O-H stretch), 2853.20, 2923.35 (Alkane C-H stretch), 1620.60 (Aromatic moiety C=C stretch), 1334.89 (Phenol O-H bend), 1046.02 (C-O-C stretch). The FTIR spectrum of Soya lecithin shows the identified peaks at 2910.67 and 2850.10 (CH_2

stretch), 1657.94 (C=O stretch), 1578.73 (C=C stretch), 1224.97 (PO_4 Asymmetric stretch band). Cholesterol shows peaks at 2931.56, 2855.77 (CH_2 stretch), and 1046.53 (C-H bend). As we compare the IR spectra of crude extract and phytosome, the phenolic O-H stretch of extract is shifted from 3326.54 to 3342.64 and 3485.66 in formulation which indicates formation of weak molecular interactions. Aromatic C=C stretch of soya lecithin (1578.73) is shifted in phytosomal formulation (1600.79). CH_2 peaks of cholesterol at 2931.56 and 2855.77 are shifted to 2923.14 and 2858.32 respectively in the phytosomes. This indicates weak intermolecular interactions due to creaction of hydrogen bonds between lipids and extract. All the corresponding peaks of drug and excipients were found in formulation which indicates no major chemical interaction in between drug and excipients.

Differential Scanning Calorimetry (DSC)

DSC is utilized in pharmaceuticals to detect purity, polymorphism, and study compatibility. It provides information on thermal events and material properties by measuring heat flow vs temperature. Thermal analysis is an essential tool for characterizing herbal extracts and plant products. The endothermic peaks at 73.66°C, 114.51°C, 276.01°C and 351.36°C suggest melting of extract, cholesterol, and soya lecithin. Melting points of cholesterol and soya lecithin are 148°C and 236°C, which is found to be missing in the spectrum, which indicates the complete incorporation of cholesterol and soya lecithin in the phytosome matrix and the formation of a chemical bond between the extract and excipients. A small exothermic peak suggests a glass transition in the formulation. Figure 7 shows the DSC thermogram of the phytosome.

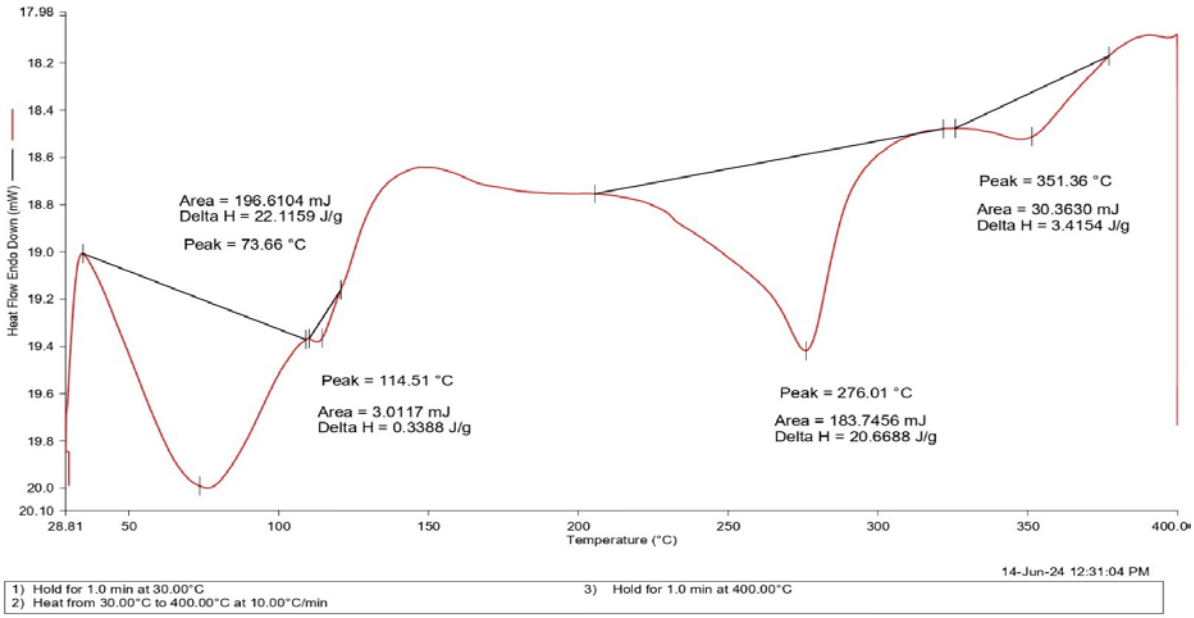

Figure 7: DSC thermogram of phytosome

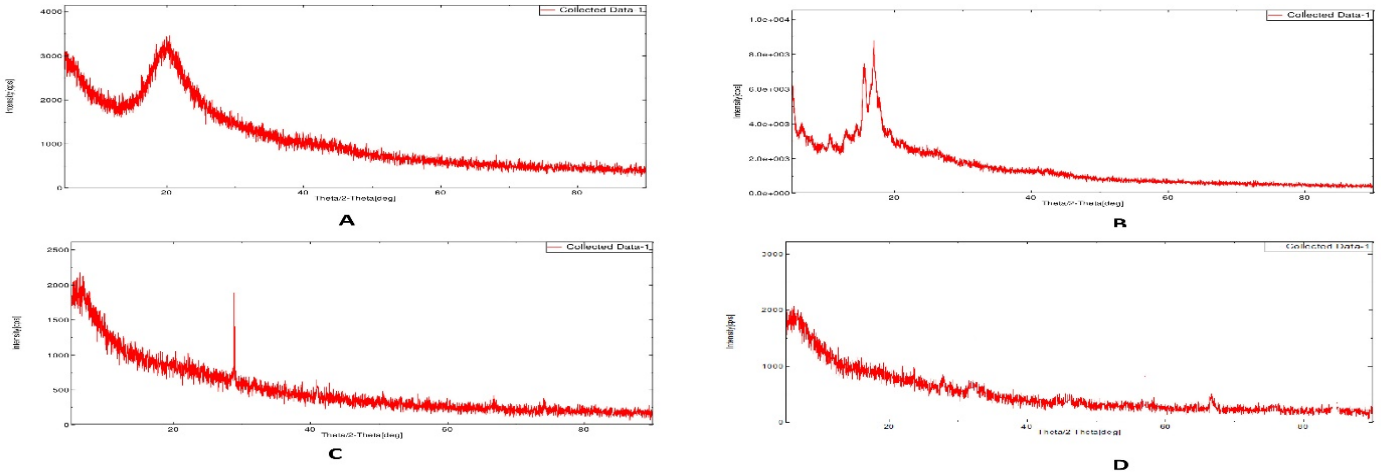

Figure 8: XRD spcrum of (A) Soya lecithin, (B) Cholesterol, (C) Extract and (D) Phytosome

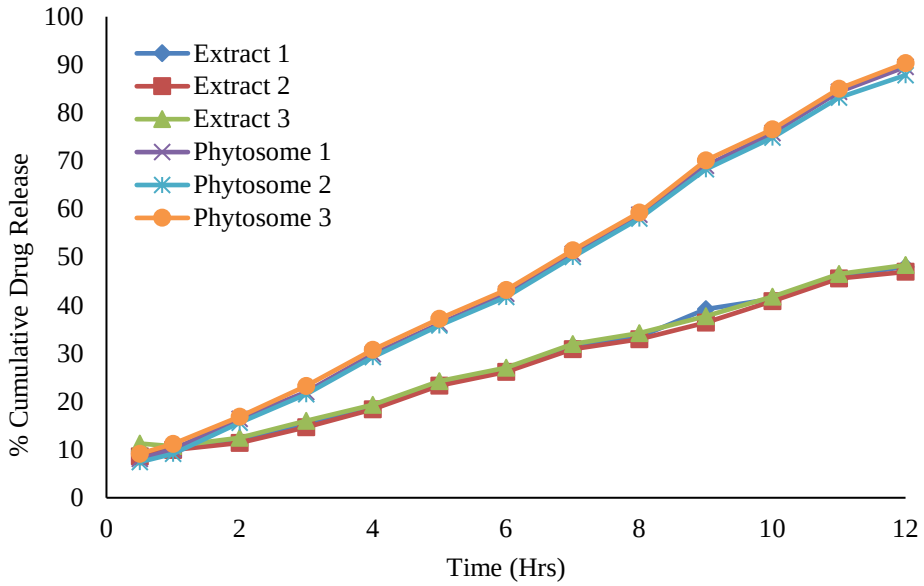

Figure 9: In-vitro dissolution profiles of Extract and Phytosome

Powder X-ray diffractometry

Powder XRD spectra of extract and excipients were performed to analyze the nature of particles. The extract shows a single peak of 201.09, while soya lecithin shows a broad peak of 192.17. This indicates their amorphous nature. Cholesterol shows three sharp peaks of height 53.31, 313.49, and 305.62. The sharpness of peaks indicates its crystalline nature. The XRD of the phytosome shows small peaks of height 51.44, 26.26, 62.97, and 35.67. Less intensity and broadness of these peaks indicate the amorphous nature of the formulation. Amorphous nature represents high solubility. XRD spectra of Lecithin, Cholesterol, Extract, and Phytosome are shown in Figure 8.

In vitro dissolution studies

The drug release mechanism from the capsules of extract and phytosome was different. In vitro dissolution studies of phytosomes showed that a fixed amount of drug was released at certain intervals. The extract showed 47.78±0.59% CDR, while the phytosome showed 89.26±1.05% CDR. This shows an increase in the dissolution of extract in phytosome formulation. High dissolution of the formulation will ultimately give high bioavailability. The dissolution profiles of pure extract and phytosomes are shown in Figure 9.

CONCLUSION

Plant-based medicines have been used for centuries and appreciated by medical professionals and patients for their better therapeutic value and lesser side effects than current medications. Phytosomes are nanocarrier dosage forms that can carry plant-based constituents to improve their solubility and bioavailability. In this study, the bioactive of *Carica papaya* seed was isolated successfully and formulated into phytosomes for the first time to enhance bioavailability. Phytochemical screening, LC-MS, and FTIR spectrophotometry confirmed the presence of phytoconstituents in the crude extract. The antidiabetic activity of the crude extract was determined by using an in-vitro antidiabetic assay that considered the aim of formulation for antidiabetic use. Phytoconstituents have low solubility and less bioavailability; phytosomes of crude extract were formulated to improve their solubility and bioavailability. The phytosomes were evaluated for several parameters such as Encapsulation efficiency, Drug content, Particle size, Zeta potential, TEM, FTIR, DSC, In vitro dissolution study, etc. From the results of evaluation parameters, it is interpreted that successful formulation of phytosomes containing bioactive from papaya seed extract. In-vitro dissolution study of phytosomes showed 89.26±1.05% CDR, and the extract showed 47.78±0.59% CDR. This indicates the dissolution of crude extract improved in phytosomal formulation. As a result, the phytosomes of the extract may enhance the bioavailability of crude extract. These phytosomes of *Carica papaya* seed extract might be used for antidiabetic activity, so further in vivo animal studies are suggested for phytosomes' antidiabetic activity.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Prashant L. Pingale examined and interpreted the experiment's design and associated data. Rima R. Patil conducted the laboratory tests and documented the observations, while Chandrashekhar D. Upasani oversaw the work and helped with the final manuscript drafting. Every author read and approved the final manuscript.

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