The Pharma Innovation Journal 2019; 8(2): 22-26

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(2): 22-26

www.thepharmajournal.com
Received: 09-12-2018
Accepted: 13-01-2019

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Preparation and evaluation of phytosomes containing ethanolic extract of leaves of *Bombax ceiba* for hepatoprotective activity

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Abstract
The plants of *Bombax ceiba* are traditionally used as home remedy in the treatment of jaundice, spleen enlargement and in some literature as Hepatoprotective activity. In the current days, the majority of the widespread diseases and nutritional disorders are treated with natural medicines. The efficiency of any herbal medication is reliant on the delivery of efficient level of the therapeutically active compound. But a harsh limitation exists in their bioavailability when administered orally or by topical applications. Phytosomes are lately introduced herbal formulations that are superior absorbed and as a result produced superior bioavailability and actions than the conformist phyto molecules or botanical extracts. The ethanolic extract of phytosome was prepared in soya lecithin. Soya lecithin has antioxidant activity. Evaluation of phytosomes for solubility study, entrapment efficiency, transition temperature, particle size and size distribution, optical microscopic study, zeta potential, transmission electron microscopy, stability studies, *in vitro* dissolution studies, and FTIR study. *In vitro* antioxidant activity of phytosomes determined by DPPH model. Combination of soya lecithin and *Bombax ceiba* can result in synergistic effect, Synergistic effect measure with free radical scavenging activity use DPPH model.

Keywords: *Bombax ceiba*, phytosomes, hepatoprotective activity, DPPH model, free radical scavenging activity

Introduction
Arrangements of plants or parts of them were extensively used in accepted medicine since ancient times and till today the use of Phyto medicine is prevalent in most of the world's population [1]. During the previous century chemical and pharmacological studies have been performed on a lot of plant extracts in sort to know their chemical composition and verify the indications of conventional medicine. It has frequently been observed that the separation and purification of the variety of components of an extract may escort to a partial loss of precise activity for the purified component. Phytosomes is a patented technology developed by a leading manufacturer of drugs and nutra ceuticals to integrate standardized plant extracts or water soluble phyto constituents into phospholipids to produce lipid compatible molecular complexes called as phytosomes and so greatly improve their absorption and bioavailability [2]. The Phytosomes process produces a tiny cell because of that the precious components of the herbal extract are sheltered from devastation by digestive secretions and gut bacteria. Phytosomes are improved able to transition from hydrophilic surroundings into the lipid-friendly surroundings of the enterocyte cell membrane and from there into the cell lastly reaching the blood [3]. Phytosomes have superior pharmacokinetic and pharmacological parameter which in result can favorably be used in the management of the acute and chronic liver disease of toxic metabolic or infective origin or of degenerative nature. It can also be used in various activities as well as in pharmaceutical and cosmetic compositions [4]. Hepatotoxicity implies chemical-drive liver damage. Liver is the more important vital organ involved in the metabolism. The liver damage ranging from subclinical Jaundice hepatitis to necro-inflammatory hepatitis, cirrhosis and carcinoma has been proved to connect with the redox imbalance and oxidative stress (OS). The synthetic drugs have been implicated in causing liver injury and it is the most frequent reason for the drugs to be withdrawn from the market, such as Troglitazone, Bromfenac, Trovafloxacin, Ebrotibine, Nefazodone and Ximedagatran etc. [5] Mechanism of liver damage is that many chemicals damage mitochondria. Its dysfunction releases excessive amounts of oxidants which in turn damages hepatic cells. Activation of some enzyme in the cytochrome p-450 system such as CYP2E1 Also leads to oxidative stress.

Injury to hepatocyte and bile duct cells escort to accretion of bile acid inside liver. This endorses further liver damage.
Bombax ceiba (Bombacaceae), is a lofty, deciduous tree. It is commonly known as silk cotton tree and broadly distributed in temperate and tropical Asia, Africa and Australia. Dissimilar parts of this plant are reported to have therapeutic potentials against different diseases such as hepatic toxicity, infections, asthenia, diabetes, polyurea and glycosuria [6-9]. Although different parts of *B. ceiba* are known to have different biological activities including antioxidant, antimicrobial, anti-inflammatory, hypotensive and hypoglycemic activity. The leaves extract of *B. ceiba* has been reported having liver protecting activity. flavonoids are multi-ring compounds generally too large to be absorbed by simple diffusion, As a result, the ability of flavonoids to cross the lipid-rich outer membrane of small intestine enterocytes is harshly limited. Phytosomes meet this challenge. Phytosomes are improved absorption, enhanced delivery and increased bioavailability of herbal extracts. Phytosomes exhibit better pharmacokinetic and pharmacodynamics profile. Phytosomes technology has been effectively used to enhance the bioavailability of many popular herbal extract [10].

Materials and Methods

Materials

The leaves of *Bombax ceiba* was collected from local area of Bhopal (MP.).

Chemical and reagents

All the other chemicals and reagents used in this study were of AR grade and were purchased from SD Fine chemicals, Mumbai.

Extraction Procedure

Defatting of plant material

Powdered plant material (leaves) *Bombax ceiba* was shade dried at room temperature. The shade dried leaves were coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was sustained till the defatting of the material had taken place.

Extraction

Initially 100 gms of material was crowded into the thimble and 2 liters of solvent used for extraction was poured into flask (Round Bottom). The soxhlet extraction was performed for 18-24 hours until the collected liquid in siphon tube shows clear. Later the extracted solvent was evaporated under reduced pressure to get dried powder of extract. Finally the percentage yields were calculated of the dried extracts [11].
In previous paper phytochemical analysis, quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoids content (TFC) result showed that ethanolic extract of *Bombax ceiba* has highest ethanolic extractive percentage compare to other extracts.

Formulation of phytosomes

Solvent evaporation technique

The specific amount of leaves extract of *Bombax ceiba* and soya lecithin were taken into a 100 ml round bottom flask and refluxed with 20 ml of acetone at a temperature 50 - 60ºC for 2 h. The mixture is concentrated to 5-10 ml to obtain the precipitate which was filtered and collected. The dried precipitate phytosomes complex was placed in amber colored glass bottle and stored at room temperature [12].

Preparation of phytosome complexes of Bombax ceiba

The different phytosome complexes F1, F2, F3, F4, F5 and F6 containing molar ratio of 1:0.5, 1:1.0, 1:1.5, 2:0.5, 2:1.0 and 2:1.5of leaves extract of *Bombax ceiba* and soya lecithin were prepared by the anti-solvent precipitation technique as mentioned in Table 1 [13, 14].

Table 1: Preparation of phytosomes

F. code	Soya lecithin: Herbal extract (Molar ration)	Acetone (ml)
F1	1:0.5	20
F2	1:1.0	20
F3	1:1.5	20
F4	2:0.5	20
F5	2:1.0	20
F6	2:1.5	20

Evaluation of phytosomes

Entrapment efficiency

Phytosomes 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 quercetin and the absorbance of supernatant for non-entrapped *Bombax ceiba* was recorded at λ max 420.0 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 phosphate buffer saline (7.4) and absorbance taken at 420.0 nm. Amount of quercetin in supernatant and sediment gave a total amount of *Bombax ceiba* in 1 ml dispersion. The percent entrapment was calculated by following formula.

$$\text{Percent Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{X } 100$$

Particle size and size distribution

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

Optical microscopic study

Phytosomes was observed under microscopy, Cippon (Japan). One drop of diluted extract-loaded nanoparticles suspension was deposited on a glass slide and it was. Excess of solution was drained off with a filter paper and then slide was allowed to dry. The sample was then examined by optical Microscopy.

In vitro drug release study

In vitro drug release of the sample was carried out using USP-type II dissolution apparatus (Paddle type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.50c and rpm of 50. Equivalent to 100 mg of phytosomes was placed in each bowl of dissolution apparatus. The apparatus was allowed to run for 10 hours. Sample measuring 5 ml were withdrawn after every 1 hour up to 10 hours using 10ml pipette. The fresh dissolution medium (37^0C) was replaced every time with the same quantity of the sample. From this take 0.5 ml and dilute

up to 10ml and take the absorbance at 420.0 nm using spectroscopy [15, 16].

Stability studies of optimize phytosome formulation

The prepared Phytosomes 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 months.

***In vitro* radical scavenging activity of phytosomes by DPPH model**

DPPH scavenging activity was measured by the spectrophotometer with slightly modification [17]. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Drug-excipient compatibility studies

The physicochemical compatibility between mixture of extract and polymer used in the research ware carried out by infrared spectral studies using Fourier Transform Infrared Spectrophotometer by using KBr dispersion method. The resultant spectrum was compared for any spectrum changes.

Results and Discussion

Absorption maxima of *Bombax ceiba* were found to be 420 nm. Standard graph showed linearity (R^2 = 0.9948) in the range of 10-60 µg/ml. The compatibility between the *Bombax ceiba* extract and excipients was evaluated using FTIR peak matching method Fig.1, 2. There was no appearance or disappearance of peaks in the drug-lipid mixture, which established the absence of any chemical interface between the drug and lipid.

Data for Particle size and% Entrapment efficiency were reported to be in the range of 217-340 nm and 50-71% respectively Table 2 & Fig.3, 4. The formulation F3 showed highest yield of 89.5%, particle size 217.90±2.45 and entrapment efficiency of 71.25% indicating drug: lipid ratio of 1:1.5 as optimum for formulation of complexes. With further increase in the lipid concentration the entrapment efficiency decreased and this indicated that the increased amount of lipid did not help in entrapping more drugs into the matrix. The best formulation F3 was subjected to structural analysis by drug release study of the formulation and UV spectroscopy.

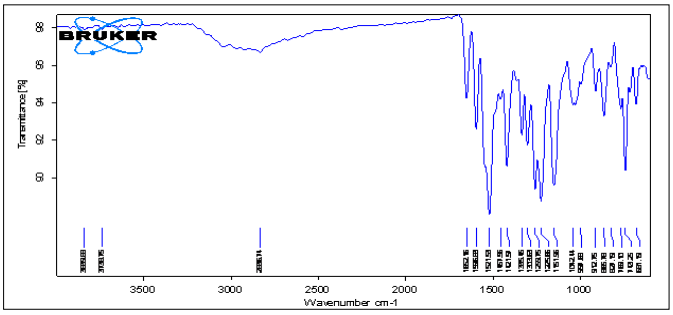

Fig 1: FT-IR spectra of *Bombax ceiba* leave extract

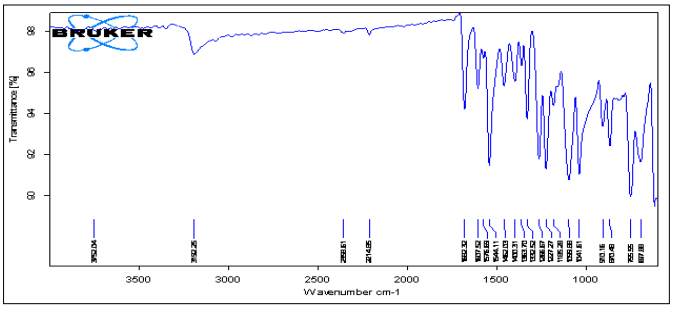

Fig 2: FT-IR spectra of phytosome

Table 2: Particle size and entrapment efficiency of extract loaded phytosomes

F. Code	Particle size	%Entrapment Efficiency
F1	250.23±1.45	56.25±2.12
F2	245.65±2.65	60.32±3.14
F3	217.90±2.45	71.25±3.52
F4	302.12±2.14	50.12±4.12
F5	340.56±1.56	54.56±3.14
F6	310.25±3.14	56.65±3.12

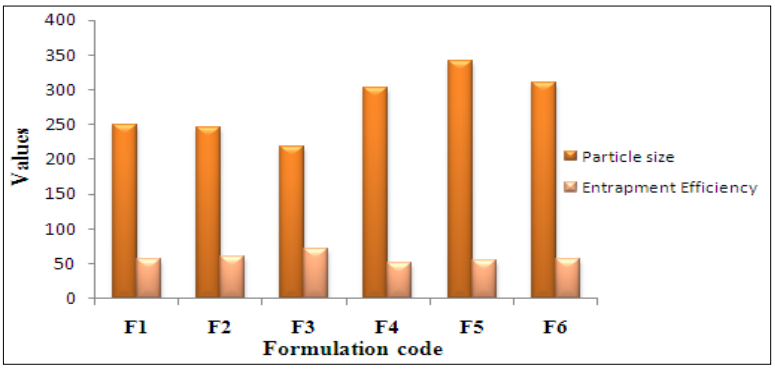

Fig 3: Particle size and entrapment efficiency of extract loaded phytosomes

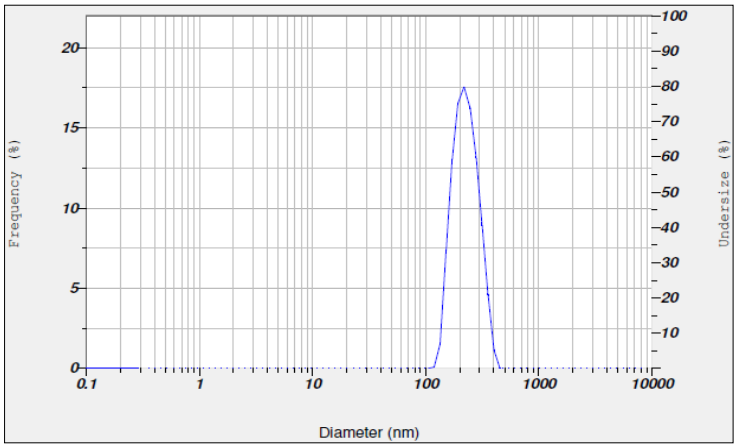

Fig 4: Particle size of optimized batch F3

In vitro dissolution study of F3 indicated that the phytosomes had extended release dissolution pattern. The phytosomes show 84.65% of release at 12 hr. Table 3 & Fig.4. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance and drug content and no significant change was observed.

Table 3: *In vitro* drug release data for F3

Time (Hrs)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ±SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	1.000	0	20.12	1.304	79.88	1.902
2	1.414	0.301	33.65	1.527	66.35	1.822
4	2.000	0.602	45.65	1.659	54.35	1.735
6	2.449	0.778	53.12	1.725	46.88	1.671
8	2.828	0.903	65.45	1.816	34.55	1.538
12	3.464	1.079	84.65	1.928	15.35	1.186

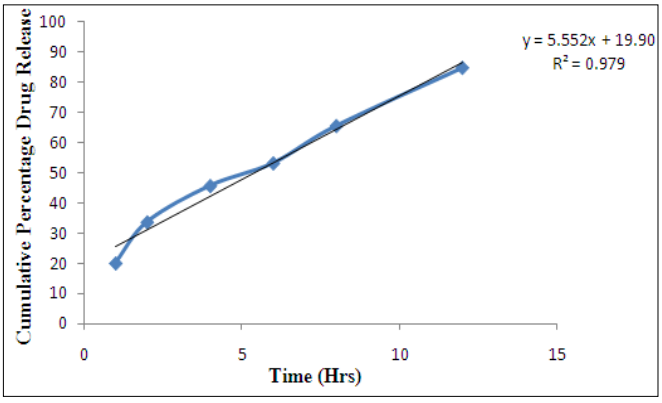

Fig 4: Zero order release of optimized batch F3

Antioxidant activity of the phytosomes was calculated through DPPH assay.% inhibition was calculated as an indicative of antioxidant potency. The higher the% inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 10 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 4 and Fig. 5.

Table 4: DPPH assay of ascorbic acid and phytosomes

S. No.	Concentration	% Inhibition	
		Ascorbic acid	Phytosomes
1	10	38.905	26.513
2	20	46.686	42.939
3	40	58.213	55.043
4	60	70.605	63.977
5	80	72.622	72.622
6	100	81.268	83.862
IC 50		26.928	38.829

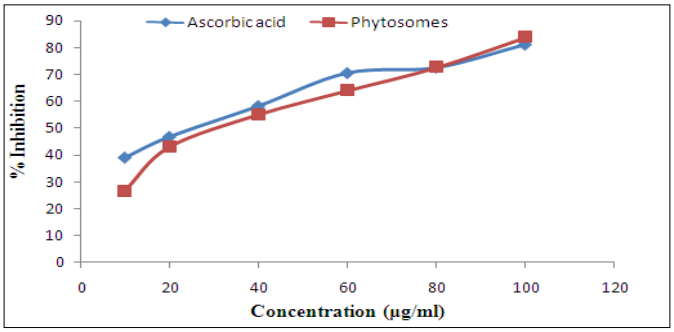

Fig 5: Graph of *In Vitro* free radical scavenging activity

Conclusion

The leaves of *Bombax ceiba* extract having liver protecting activity. Phytosomes study can increase therapeutic efficacy, decrease the frequency of administration. Phytosome formulated with solvent evaporation method. The different formulation prepare 1:0.5, 1:1.0, 1:1.5, 2:0.5, 2:1.0 and 2:1.5 ratio. The best formulation (F3) selected ratio1:1.5 for final formulation. *In vitro* dissolution study of phytosome extended release pattern show 12 hr, 84.65% release. The synergistic effect determined by free radical scavenging activity of *Bombax ceiba*-phytosome using DPPH model. In conclusion, phytosome successfully prepared and encapsulated. It show extended release pattern with enhanced free radical scavenging activity. *Bombax ceiba* plant shows hepatoprotective activity as well as they are traditionally used in the treatment of diabetes.

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