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Research Article

FORMULATION AND CHARACTERIZATION OF PHYTOSOMES OF *SARACA ASOCA*

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Abstract:

The objective of the study was to develop a novel Phytosome formulation of *Saraca Asoca* by incorporation of phospholipids, for the improved permeability, solubility and better physical characteristics. The formations of phytosomes were analyzed for SEM, measurement of particle size, drug content, drug entrapment efficiency, Percentage yield, In-vitro drug release studies and also the release kinetics of *Saraca Asoca* phytosomes complex. The drug content, drug entrapment efficiency, SEM, measurement of particle size and other In-vitro drug release studies were resulted as anticipated. The *Saraca Asoca* phytosomes were found to show better solubility and compatibility with the excipients, it is concluded that *Saraca Asoca* phytosomes has better physical characteristics and improved permeability, solubility than that drug to overcome ability to cross lipid-rich biological membranes and which results in increase oral bioavailability.

Keywords: *Saraca Asoca*, Phytosome complex, Drug content, Drug entrapment efficiency, Percentage yield, In-vitro drug release.

Introduction:

Phytosome

The term “phyto” means plant while “some” means cell-like. Phytosome is a novel emerging technique applied to phyto-pharmaceutical which contains phytoconstituents of herbal extract surrounds and bound by lipid. Since ancient times the therapeutic uses of traditional medicines and phyto-medicines have proved very popular for health maintenance by various means. The advancement in the field of herbal drug delivery started recently with the aim to manage human diseases efficiently. Every nation is seeking health care beyond the traditional boundaries of modern medicine; turning to self medication in the form of herbal remedies. Most of bioactive constituents of phyto-medicines are water soluble molecules (e.g. Phenolics, glycosides, flavonoids etc.). However, water soluble phytoconstituents are limited in their effectiveness because they are poorly absorbed when taken orally or when applied topically.

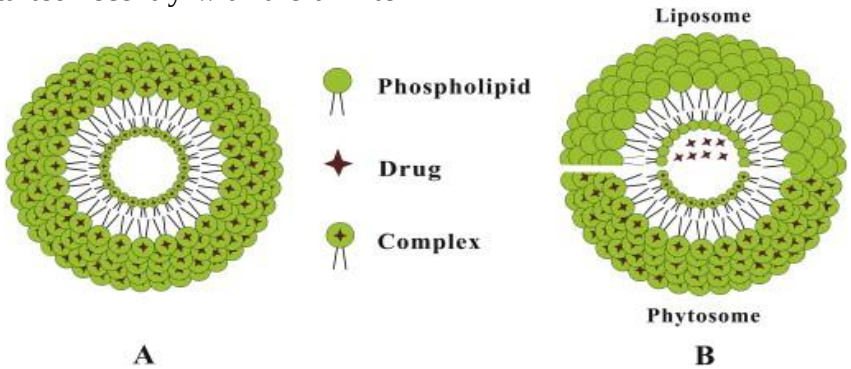

Figure 1.1: Structure of phytosomes

Many approaches have been developed to improve the oral bioavailability, such as inclusion of solubility and bio availability enhancer, structural modification and entrapment with the lipophilic carriers and thus extensive research in the field of herbal drug delivery systems as a means of improving the therapeutic indices of drugs is inevitable. The use of formulation technology to deliver herbal products and drugs by improved absorption and, as a consequence, produce better results than those obtained by conventional herbal extracts. Phytosome are not liposome; structurally the two are distinctly different. The phytosome is a unit of a few molecules bonded together, while liposome is an aggregate of many phospholipid molecules and encloses other phyto-active molecules but without specially bonding to them. Phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, without compromising nutrient safety.

The term 'Phyto' means plant while 'Some' means cell-like. Phytosome is vesicular drug delivery system in which phytoconstituents of herb extract surround and bound by lipid (one phyto-constituent molecule linked with at least one phospholipid molecule). Phytosome protect valuable component of herbal extract from destruction by digestive secretion and gut bacteria and because of which they shows better absorption which produces better bioavailability and improved pharmacological and pharmacokinetic parameters than conventional herbal extract. The advantages and disadvantage of phytosomes are summarized below.

Advantages

1 There is a dramatic enhancement of the bioavailability of botanical extracts due to their complexation with phospholipid and improved absorption in the intestina l tract.

2 They permeate the non-lipophilic botanical extract to allow better absorption from the intestinal lumen, which is otherwise not possible

3 The formulation of Phytosome is safe and the components have all been approved for pharmaceutical and cosmetic use.

4 They have been used to deliver liver protecting flavonoids because they can be made easily bioavailable by phytosomes. In addition to this, Phosphatidylcholine is also hepatoprotective and so provides a synergistic effect for liver protection

5 This technology offers cost effective delivery of phytoconstituents and synergistic benefits when used as functional cosmetics to protect the skin against exogenous or endogenous hazards in normal as well as stressful environmental conditions

6 They can be also used for enhanced permeation of drug through skin for transdermal and dermal delivery

7 These are platform for the delivery of large and diverse group of drugs (peptides, protein molecules).

8 The vesicular system is passive, non-invasive and is available for immediate commercialization

9 Phosphatidylcholine, an essential part of the cell membrane used in phytosome technology, acts as a carrier and also nourishes the skin

10 There is no problem with drug entrapment during formulation preparation.

11 Also, the entrapment efficiency is high and moreover predetermined; because the drug itself forms vesicles after conjugation with lipid.

12 They offer a better stability profile because chemical bonds are formed between the phosphatidylcholine molecules and Phytoconstituents.

Properties of Phytosomes

Physico-chemical properties

- Phytosome are prepared by reaction of stoichiometric amount of phospholipid with the standardized plant extracts as substrate. The spectroscopic data reveals that the phospholipid substrate interaction is due to the formation of hydrogen bond between the polar head (i.e., phosphate and ammonium group) and the polar functionalities of the substrate.
- The size of Phytosome varies from 50 nm to a few hundred μm. Phytosome when treated with water assumes a micellar shape resembling liposome and photon correlation spectroscopy (PCS) reveals this liposomal structures acquired by phytosome.
- The H1NMR and C13 NMR data deduced that the fatty chain gives unchanged signals both in free phospholipid and in the complex, which indicates that long aliphatic chains are wrapped around the active principle producing lipophilic envelope
- The complexes are often freely soluble in aprotic solvents, moderately soluble in fats, insoluble in

water and relatively unstable in alcohol. But the phytosomes of certain lipophilic phytoconstituents like curcumin has shown increase in water solubility upon complexation with phospholipid.

MATERIAL AND METHOD

Collection of Plant material

Aerial parts of *Saraca asoca* was collected from Vindhya Herbal Nursery Bhopal (M.P), India in month of September (2020).

Extraction of Plant Material

50 gm.of *Saraca asoca* dried plant material were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 80:20). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Determination of Percentage yield

Calculation of percentage yield

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

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Qualitative phytochemical analysis of plant extract The leaves of *Saraca Asoca* extract obtained were subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from of *Saraca Asoca* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

In-vitro antioxidant activity using DPPH method

$$\textit{Calculation of \% Reduction} \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Formulation development of phytosomes

Preparation of phytosomes

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

Table 1: Different formulations of phytosomes

Formulation	Ratio of Phospholipids and Cholesterol	Extract Concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:1	1	25
F2	1:2	1	25
F3	**2:1**	**1**	**25**
F4	2:3	1	25
Optimization of Drug Concentration			
F5	2:1	0.5	25
F6	**2:1**	**1.0**	**25**
F7	2:1	1.5	25
F8	2:1	2.0	25
Optimization of solvent concentration			
F9	2:1	1.0	10

F10	**2:1**	**1.0**	**25**
F11	2:1	1.0	50
F12	2:1	1.0	75

Characterization of prepared Phytosome

(a) Microscopic observation of prepared Phytosome

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the optimized Phytosome formulation.

(b) Drug Excipient compatibility study by FT-IR

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

(c) Entrapment efficiency

Phytosome 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 flavonoids and the absorbance of supernatant for non entrapped *Saraca asoca*. 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 0.1 N HCl and absorbance taken at 420.0 nm. Amount of quercetin in supernatant and sediment gave a total amount of *Saraca asoca*. in 1 ml dispersion. The percent entrapment was calculated by following formula.

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

(d) 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.

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

(f) *In-vitro* dissolution rate studies

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37 ± 0.5^0C and 75 rpm. 10 mg of prepared phytosomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37^0C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy.

Table 2: Interpretation of diffusional release mechanisms

Release exponent (*n*)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5<n<1.0$	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

(g) Stability studies of optimize phytosomes 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. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance and drug content.

RESULTS AND DISCUSSION

Result of percentage yield of extract

The crude extracts so obtained after the maceration process, extract were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using hydroalcoholic solvent is depicted in the table 3.

Table 3: Result of percentage yield of extract of *Saraca asoca*

S. No.	Solvents	Percentage Yield (%)
1.	Hydroalcoholic	4.08

Results of phytochemical Testing

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of sample. Small amount of extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 4.

Table 4: Result of phytochemical screening of *Saraca asoca* extract

S. No.	Constituents	Hydroalcoholic Extract
1.	**Alkaloids**	
	Dragendorff's Test:	-ve
	Wagner's test:	+ve
	Mayer's Test:	-ve
2.	**Carbohydrate**	
	Molisch's test:	+ve
	Benedict's test:	-ve
	Barfoed's test:	-ve
	Anthrone test:	-ve
3.	**Glycosides**	
	Legal's test:	-ve
	Baljet's test:	-ve
	Borntrager's test:	-ve
	Keller Kiliani test:	-ve
4.	**Saponins**	
	Froth test:	+ve
5.	**Flavonoids**	
	Lead Acetate Test	+ve

6.	**Proteins & Amino Acids** Biuret's test: Ninhydrin's test: Xanthoprotein test: Millon's test:	 -ve -ve -ve -ve
7.	**Phenol** Ferric Chloride Test:	 +ve
8	**Resin** HCLExtract solution in dil. Water	 -ve
9	**Fixed Oil** Filter Paper Test	 +ve

From the results obtained it is clear that the *Saraca asoca*. plant shows the presence of Alkaloids, Carbohydrate, Saponins, Fixed Oil, Phenol, Flavonoids were found present in extracted with Hydroalcoholic solvent using maceration procedure.

Results of Estimation of Total flavonoid content

Table 5: Total flavanoid content of Hydroalcoholic extract of *Saraca asoca*

S. No.	*Saraca asoca*	
1.	Total flavonoid (QE) (mg/100mg)	9.33±0.5

Results of antioxidant activity using DPPH method

Table 6: % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

S. No.	**Concentration (µg/ml)**	**% Inhibition**	
		Ascorbic acid	**Hydroalcoholic extract**
1	10	44.65	21.19
2	20	48.62	25.26
3	40	65.34	29.45
4	60	69.65	34.25
5	80	77.41	38.22
6	100	84.13	41.37
IC 50		17.681	84.425

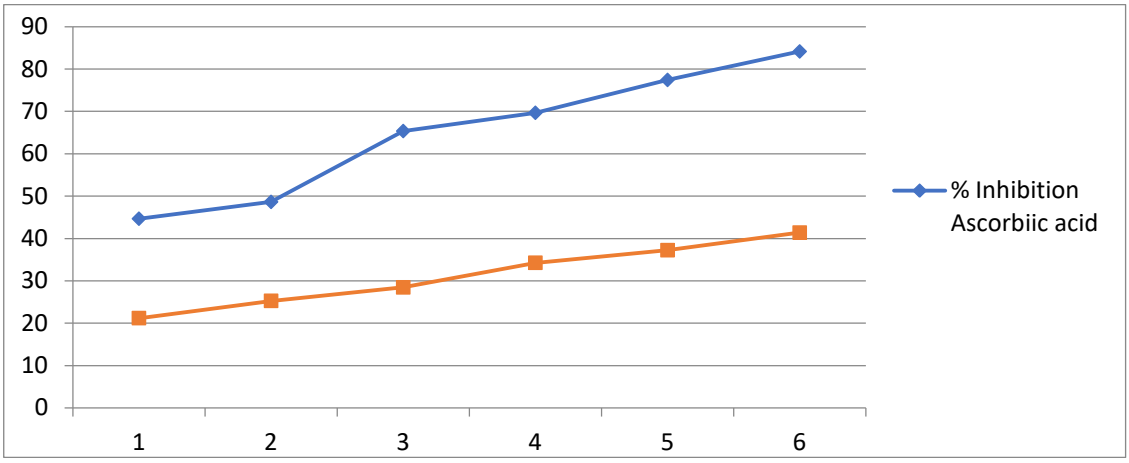

Figure 2: % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

Characterization of phytosomes of hydroalcoholic extract *of Saraca asoca*

Microscopic observation of prepared phytosomes

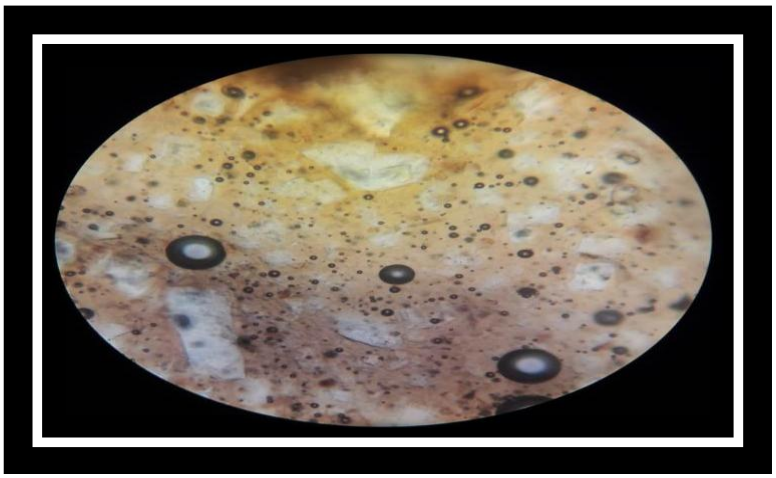

Figure 3: Microscopic observation of optimized batch F10

Result of Drug-Excipient compatibility study

The spectrum of extract and phytosomes was authenticated by FTIR spectroscopy. The presences of characteristic peaks associated with specific structural characteristics of the drug molecule were noted. Various peaks of the drug are shown in Figure and the wave numbers are listed in Table.

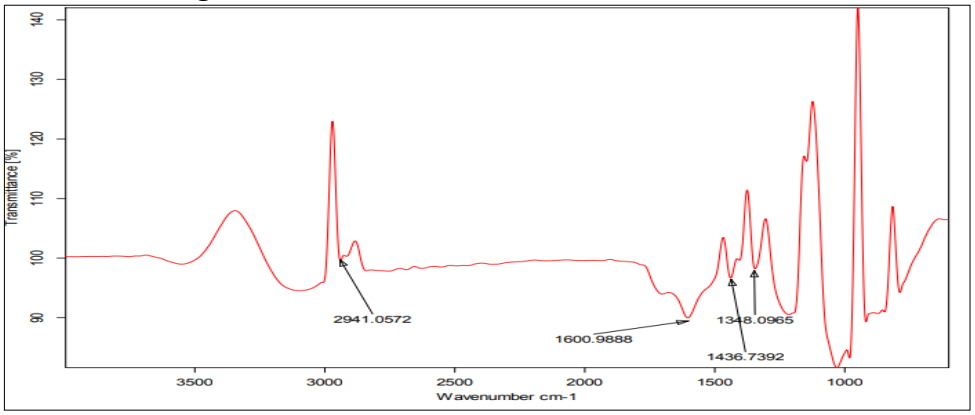

Figure 4: FT-IR spectra of hydroalcoholic extract

Table 7: Interpretation of FT-IR spectrum

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

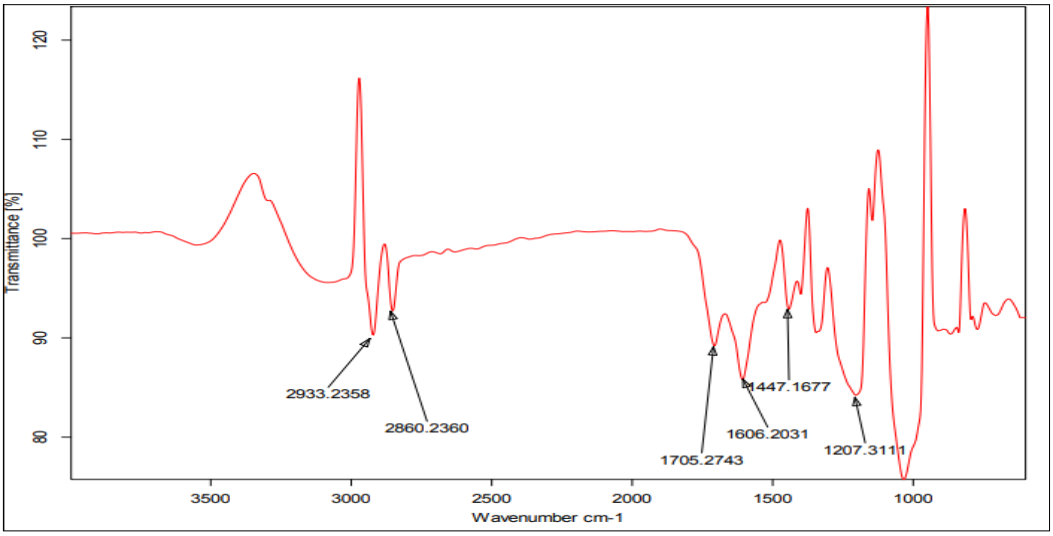

Figure 5: FT-IR spectra of prepared phytosomes formulation

The appearance or disappearance of peaks and/or the shift of their positions are often indications of interactions such as hydrogen bonding. The IR spectra of extract, Fig.4,5 shows stretching vibrations at 1600.9880 cm^{-1} attributed predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Infrared of extract show stretching vibration at 2941.0572cm^{-1} due to O-H groups, C=C aromatic stretching vibration at 1436.7392cm^{-1}. When the data obtained from FTIR spectra is compared with the spectra studied it was observed that there are similar peaks for functional groups in Phytosomes.

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

Entrapment efficiency and particle size analysis

Entrapment efficiency is an important parameter for characterizing phytosomes. In order to attain optimal encapsulation efficiency, several factors were varied, including the concentration of the lipid, concentration of drug and concentration of alcohol. The entrapment efficiency of all the prepared formulations is shown in Table 9. The entrapment efficiency of the phytosomes was found in the range of 50.16±0.45 to 75.15±0.12%.

Particle size of all formulations found within range 312.95±0.25-435.65±0.18 nm. Concentration of lipid has shows significant impact on size of phytosomes. Formulation F10 was found best one which is further evaluated for drug release study, transmission electron microscopy (TEM), and stability studies.

Table 8: Particle size and entrapment efficiency of drug loaded phytosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	411.39±0.45	50.16±0.45
F2	435.65±0.18	52.32±0.36
F3	399.16±0.25	51.98±0.21
F4	345.15±0.21	52.89±0.45
F5	411.25±0.41	55.65±0.58
F6	364.58±0.25	62.83±0.78
F7	376.89±0.51	65.58±0.32
F8	409.23±0.32	61.64±0.91
F9	329.56±0.65	64.98±0.58
F10	**312.95±0.25**	**75.15±0.12**
F11	423.56±0.41	62.21±0.39
F12	424.45±0.23	61.74±0.28

Average of three determinations (n=3)

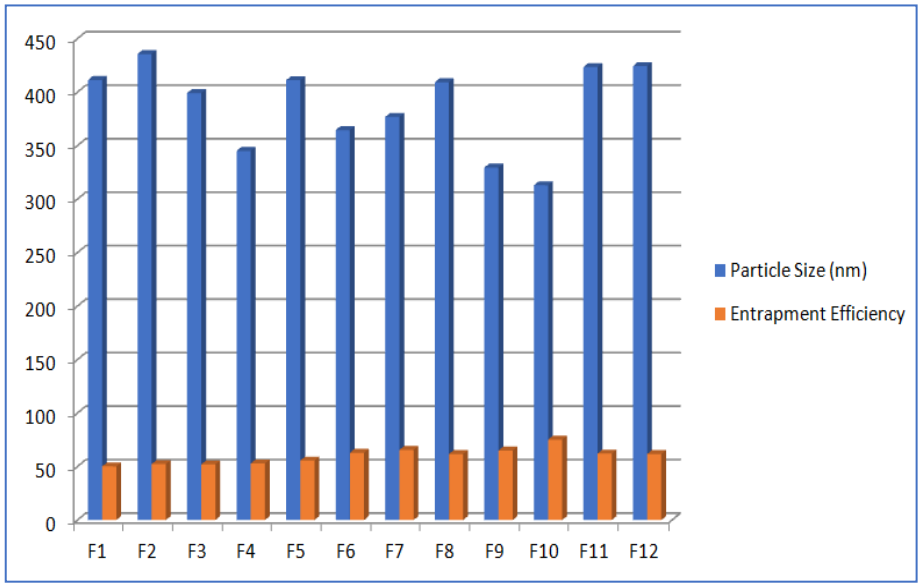

Figure 6: Graph of Particle size and entrapment efficiency

Transmission Electron Microscopy (TEM): TEM is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail even as small as a single column of atoms, which is thousands of times smalle r than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEM characterization revealed that the Phytosomes are spherical in shape. However, some variation in size distribution was observed in the TEM image, which might be attributed to an uncontrolled charge neutralization process involved between oppositely charged chains occurring during the formation of phytosomes.

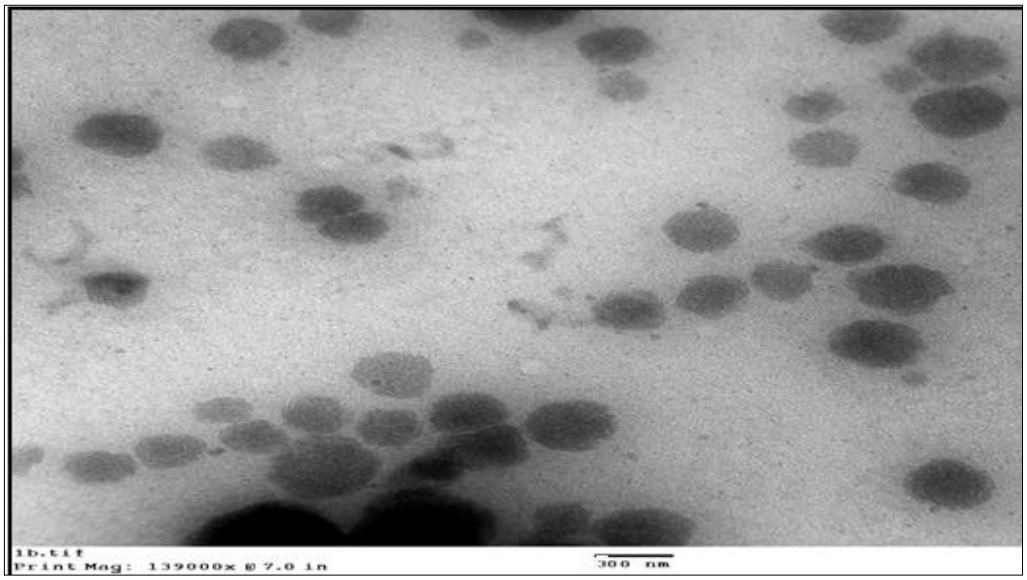

Figure 7: TEM image of phytosomes

***In vitro* drug release study of prepared Phytosomes formulation**

Table 9: Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.910	0.894	0.968	0.982

When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was maximum i.e. 0.968 hence indicating drug release from formulations was found to follow Higuchi release kinetics.

Results of stability studies

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

SUMMARY AND CONCLUSION

The phytochemical screening of this investigation attested the presence of several secondary metabolites with known biological antioxidant activities. Hydroalcoholic extract of aerial parts of *Saraca asoca* was possessing antioxidant activity due to the presence of flavonoids constituent. Flavonoids content was expressed as milligrams equivalent of Quercetin per 100 milligrams of dry extract (mg QE/100mg). Total flavonoid contents are shown in Table 5. From these results, Hydro-alcoholic extract showed flavonoid compounds (9.33±0.5 mg QE/100mg). Different formulation of Phytosomes were prepared using different amount of phospholipids: cholesterol and extract and were evaluated for Drug Excipient compatibility study, Entrapment efficiency and particle size analysis, High performance liquid chromatography, Transmission Electron Microscopy (TEM), In vitro drug release study of prepared Phytosomes formulation and Stability study. The appearance or disappearance of peaks and/or the shift of their positions are often indications of interactions such as hydrogen bonding. The IR spectra of extract, Fig.4,5 shows stretching vibrations at 1600.9880 cm^{-1} attributed predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Infrared of extract show stretching vibration at 2941.0572cm^{-1} due to O-H groups, C=C aromatic stretching vibration at 1436.7392cm^{-1}. When the data obtained from FTIR spectra is

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