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FORMULATION, DEVELOPMENT AND EVALUATION OF PHYTOSOMES OF *SWERTIA PERENNIS*

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ABSTRACT

The aim of the present study was performed, Extraction of plant material using hydroalcoholic extract, Phytochemical analysis of *Swertia perennis* L and Formulation, development and evaluation of phytosomes. The phytochemical screening of this investigation attested the presence of several secondary metabolites with known biological antioxidant activities. Hydroalcoholic extract of aerial parts of *Swertia perennis* 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 7.4. From these results, Hydro-alcoholic extract showed flavonoid compounds (1.021 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, In vitro drug release study of prepared Phytosomes formulation. Entrapment efficiency is an important parameter for characterizing phytosomes. When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was maximum i.e. 0.964 hence indicating drug release from formulations was found to follow Higuchi release kinetics.

Keywords: Swertia Perennis, Phytochemical, antioxidant, phytosomes, evaluation.

INTRODUCTION

Herbal drug treatments had been essential source of number one healthcare in everywhere in the global. From historic instances, plants had been catering as wealthy supply of effective and secure drug treatments. About 80 % of worldwide populations are nevertheless dependent on traditional drugs. Herbal medicines are completed, categorised medicinal products that comprise as lively elements, aerial or underground a part of vegetation or other plant materials, or combination thereof, whether or not within the crude country or as plant arrangements. Medicines containing plant substances blended with

chemically defined energetic substances, together with chemically described remoted elements of plant life aren't considered to be herbal drug treatments[1.]

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.

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[2]. 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)[3]. Phytosome protect valuable component of herbal extract from destruction by digestive secretion and gut bacteria and because of which they show better absorption which produces better bioavailability and improved pharmacological and pharmacokinetic parameters than conventional herbal extract. *Swertia chirata* is known as Chirayata in India. *S. chirata* is used as antipyretic, anthelminitic, antiperiodic, cathartic and in asthma and leucorrhoea in Ayurveda and as harsh, analeptic, stomachic, mitigate inflammation, relaxing to pregnant uterus and never ending fevers. It is a remedy for ulcers, gastrointestinal diseases, skin diseases, cough, hiccup, liver and kidney diseases, neurological disorders and urinogenital tract disorders. Also used as purifier of Breast milk, and as a laxative and carminative. The aim of the present study was performed, Extraction of plant material using hydroalcoholic extract, Phytochemical analysis of *Swertia perennis* L and Formulation, development and evaluation of phytosomes.

Materials and Methods:

Materials:

Seeds of *Swertia perennis* was collected from local area of Bhopal (M.P.) Plant material (Seeds) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Methods

Extraction procedure[4-5]

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs:

Defatting of Plant Material

Powdered aerial parts of *Swertia perennis* L. were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

42.5g. of *Swertia perennis* L. dried plant material were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 70:30). 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 tests

The extracts obtained by solvent extraction were subjected to various qualitative tests to detect the presence of plant constituents.

Quantitative estimation of phytoconstituents[6]

Total flavonoid content

Principle: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extracted dissolve in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

***In-vitro* antioxidant activity using different methods**[7]

DPPH method

DPPH scavenging activity was measured by the spectrophotometer. 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 and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

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

Formulation development of phytosomes[8]

Preparation of phytosomes

The complex was prepared with phospholipids: Cholesterol and *Swertia perennis* L. 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

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.

Entrapment efficiency[9]

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 *Swertia perennis* L. 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 *Swertia perennis* L. 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[10]

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.

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.

RESULTS AND DISCUSSION

The phytochemical screening of this investigation attested the presence of several secondary metabolites with known biological antioxidant activities shown in Table 3. Hydroalcoholic extract of aerial parts of *Swertia perennis* 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). From these results, Hydro-alcoholic extract showed flavonoid compounds (1.021 mg QE/100mg) shown in Table 4. 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, In vitro drug release study of prepared Phytosomes formulation. 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 6. The entrapment efficiency of the phytosomes was found in the range of 48.56±0.45 to 74.65±0.32%. Particle size of all formulations found within range 313.25±0.36-436.65±0.36nm. Concentration of lipid has shows significant impact on size of phytosomes. When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was maximum i.e. 0.964 hence indicating drug release from formulations was found to follow Higuchi release kinetics.

Table No. 2: Result of percentage yield of extract of *Swertia perennis* L

S. No.	Solvents	Percentage Yield (%)
1.	Hydroalcoholic	5.3

Table No. 3: Result of phytochemical screening of *Swertia perennis* L extract

S. No.	Constituents	Hydroalcoholic Extract
1.	**Carbohydrate**	
	Molisch's test:	-ve
	Benedict's test:	-ve
	Barfoed's test:	-ve
	Anthrone test:	+ve
2.	**Alkaloids**	
	Dragendorff's Test:	-ve
	Wagner's test:	-ve
	Mayer's Test:	-ve
	Hager's 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**	
	Shinoda test:	+ve
6.	**Proteins & Amino Acids**	
	Biuret's test:	+ve
	Ninhydrin's test:	+ve
	Xanthoprotein test:	-ve
	Millon's test:	-ve
	Lead Acetate test:	-ve
7.	**Phenol**	
	Ferric Chloride Test:	-ve

Table No. 4: Total flavanoid content of Hydroalcoholic extract of *Swertia perennis* L.

S. No.		*Swertia perennis* L
1.	Total flavonoid (QE) (mg/100mg)	1.021

Table No. 5: % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	25.14
2	20	48.62	29.36
3	40	65.34	33.14
4	60	69.65	39.65
5	80	77.41	41.22
6	100	84.13	45.77
	IC 50	17.681	116.425

Table 6: Particle size and entrapment efficiency of drug loaded phytosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	412.32±0.45	48.56±0.45
F2	436.65±0.36	52.32±0.36
F3	398.56±0.25	49.98±0.21
F4	385.65±0.21	52.23±0.45
F5	412.25±0.47	56.65±0.58
F6	365.58±0.65	62.23±0.78
F7	378.89±0.58	66.58±0.32
F8	412.23±0.32	61.74±0.74
F9	335.56±0.65	63.98±0.65
F10	**313.25±0.36**	**74.65±0.32**
F11	425.56±0.41	62.25±0.41
F12	422.45±0.23	61.14±0.25

Average of three determinations (n=3)

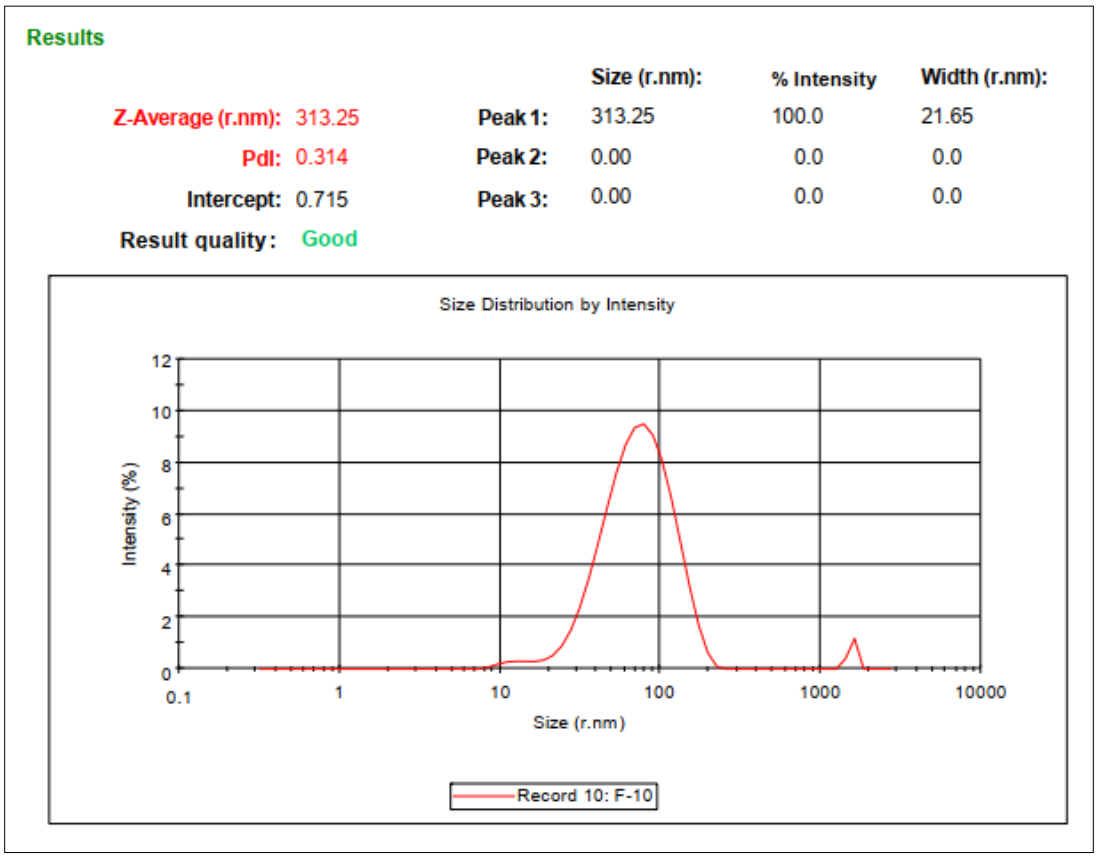

Figure 1: Particle size of optimized batch F10

Table 7: *In-vitro* drug release data for optimized formulation F10

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	23.36	1.368	76.64	1.884
1	1	0	36.65	1.564	63.35	1.802
2	1.414	0.301	45.58	1.659	54.42	1.736
4	2	0.602	56.65	1.753	43.35	1.637
6	2.449	0.778	72.23	1.859	27.77	1.444
8	2.828	0.903	85.56	1.932	14.44	1.160
12	3.464	1.079	98.89	1.995	1.11	0.045

Table 7: 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	0.896	0.964	0.987

CONCLUSION

In conclusion, in this study, the combined hydroalcoholic extract of *Swertia perennis* in ratio of 2:1:1 found to exhibit significant results. Phytosomes has better physical characteristics than that of extract. *In-vitro* studies revealed that phytosomes showed control release of phytoconstituents. Hence, phytosomal formulation of this herbal drug combination can be used for clinical application to enhance the therapeutic effect.

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