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RESEARCH ARTICLE

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FORMULATION AND EVALUATION OF PHYTOSOMES LOADED WITH *TABERNAEMONTANA DIVARICATA* LEAF EXTRACT

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Abstract

The objective of the present study was to prepare phytosomal formulation loaded with aqueous extract of *Tabernaemontana divaricata* fruits. The extraction of leaf powder was done by maceration technique using distilled water with an extraction yield of 7.97 %. The extract was found to contain alkaloids, and flavonoids and the total phenolic content was found to be 19.4 ± 0.623 %w/w. Phytosomes of the extract were prepared by solvent evaporation method using lecithin as the lipid molecule. The particle size of the phytosomes was from 596 nm to 1365 nm in size. The phytosomes were visible as rigid, almost spherical vesicles in SEM image. The surface of the phytosome vesicles was found to be regular and smooth. The point prediction suggested level 3 (0.3 g) of lipid concentration and level 1 (0.1 g) of extract concentration to present the lowest particle size. Sharp and distinct endothermic peaks in DSC revealed the formulation of stable phytosomes due to molecular interactions between the extract and lecithin. The phytosomes were found to possess good antioxidant action in DPPH assay with reference to the extract suggesting that phytosomal preparation retained the properties of the extract.

Keywords

Phytosome, Tabernaemontana divaricata, antioxidant, lecithin, solvent evaporation

Introduction

India is among the biggest producer of herbal medicines. Be that as it may, because of complex nature of herbal extract, expansive dosages, poor bioavailability and dose frequency, there use in present day therapeutic framework is restricted. The requirements of novel drug delivery system of herbal extracts than conventional herbal extracts are as follows: to enhance the bio-availability of herbal extracts, and to decrease the dose of herbal extracts.

Phytosomes are known contain the bioactive phytoconstituents of herb extract bounded by lipids and are developed by incorporating standardized plant extract or water soluble bioactive plant constituent into phospholipids to make lipid compatible molecular complex called phytosomes and so progress their absorption and bioavailability[1-7].

Tabernaemontana divaricata has been known to contain flavonoids and phenolic compounds that act as strong antioxidants owing to their free radical scavenging potential. The plant is used in the form of oil, gum, or extracts for cure of several diseases[8].
The objective of this work was to formulate the phytosomal system as an efficient method for oral delivery of *Tabernaemontana divaricata* leaf hydroalcoholic extract and to assess its antioxidant potential

Material and Methods

Collection and authentication of Plant material

The leaves of *Tabernaemontana divericata* were collected from the locality of Bhopal district of Madhya Pradesh. The leaves were washed with 0.2% hypochlorite solution, rinsed with water, shade dried and powdered using a blender at low speed. The leaf powder was stored in air tight container until taken for use.

Extraction and phytochemical screening of Plant Material[9,10]

An accurately weighed quantity of 50 g of powdered plant material was taken in a 2 L glass jar. The jar was filled with 1000 mL of distilled water and macerated for 24 h with intermittent shaking for first 6 h and allowing standing for 18 h. The macerate was filtered and the filtrate was evaporated on water bath. The thick syrupy residue obtained was subjected to rotary evaporation to remove all solvent. The dried extract was kept in desiccator until used for various tests. The extract was tested for various classes of plant metabolites using the color tests.

Total Phenolic content[11]

One gram of the nutraceutical powder was added to 15 ml of methanol (50% v/v in water) and extracted by maceration for 2h, then filtered and made up the volume with methanol (50% v/v in water) in volumetric flask upto 100 ml. One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/v) Na_2CO_3 was added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the sample was measured at 765 nm against blank, i.e., distilled water. Gallic acid was used as standard.

Preparation and optimization of Phytosomes by solvent evaporation method[12]

The specific amount of fruit extract of *Tabernaemontana divericata* and soya lecithin (Table 1) were taken into a 100 mL round bottom flask and refluxed with 30 mL of ethanol at a temperature 40 – 50°C for 4 h. The mixture was concentrated to 5-10 ml to obtain the sticky precipitate which was lyophilized to obtain the phytosomes. The dried phytosomes complex was placed in amber colored glass bottle and stored in refrigerator.

Table 1 Batch processing for phytosome preparation

Formulation Code	Lecithin	Extract	Ethanol (mL)
TP 1	0.3	0.1	30
TP 2	0.2	0.3	30
TP 3	0.2	0.1	30
TP 4	0.1	0.2	30
TP 5	0.1	0.1	30
TP 6	0.3	0.3	30
TP 7	0.2	0.2	30
TP 8	0.1	0.3	30
TP 9	0.3	0.2	30

Evaluation of phytosomes[13]

Visualization

Visualization of phytosomes was accomplished by utilizing scanning electron microscopy. Scanning electron microscopy has been utilized to decide particle size estimate appropriation and surface morphology of the complex. The samples were sputter-covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive SEM (Hitachi-S 3400N) and watched for morphology at voltage of 15.0 kV.

Particle size and size distribution

The particle size (z-average) and size distribution of the prepared phytosomes was calculated from the auto correlation function of the intensity of light scattered from the particles expecting a circular type of particles using Malvern Zeta sizer.

Differential scanning calorimetry

The thermograms were obtained for the phytosome and lecithin to ensure compatibility. Each sample was heated in the range of temperature 25°C to 300°C at a heating rate of 5°C per minute. The thermograms were observed for enthalpy changes, appearance/vanishing of peaks, and changes to a peaks onset time, shape, and relative area.

Anti-oxidant activity

DPPH radicals scavenging activity[14]

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Separately, 1mM solution of DPPH, phytosome and extract solution (50-250 µg/mL) were prepared in ethanol. 1.5ml of the phytosome or extract was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(A_{con} - A_{test})}{A_{con}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the sample of the extracts.

Results and Discussion

Extraction and Phytochemical Screening

The yield of the extract was calculated and the extract was qualitatively screened for various classes of phyto-constituent according to the methods reported in previous sections and the results obtained thereof are presented in Table 6.2. The extract was dark green in color and obtained in a yield of 7.97 %. The phytochemical screening revealed the presence of alkaloids and flavonoids in the extract.

Total Phenolic Content

Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be y = 0.0046x + 0.0037 (Figure 1). The total phenolic content in extracts, expressed as percent w/w.

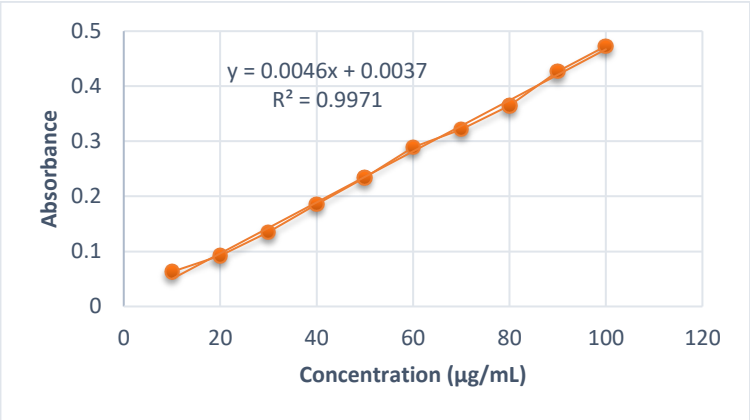

Figure 1 Calibration curve of gallic acid

The total phenolic content in the aqueous extract of *Tabernaemontana divaricata* was found to be 19.4 ± 0.623 %w/w.

Preparation of phytosomes

The phytosomes loaded with *Tabernaemontana divaricata* fruit extract were prepared using solvent evaporation method. In this technique, the phytoconstituents or extract and the lipid (lecithin) are kept in a flask containing organic solvent. This reaction mixture is kept at an optimum temperature usually 40^{o}C for specific time period to attain maximum drug entrapment in the phytosomes formed. The organic solvent is then removed using rotary evaporator.

Optimization of the phytosomes

Particle size was used as the response factor to assess the best combination of the lipid and extract in preparing phytosomes. The objective was to achieve the minimum possible size. The particle size of the nine prepared formulations was obtained and subjected to ANOVA analysis in order to generate the equation that could predict particle size optimally.

The particle size for each batch of phytosomes was determined using zeta sizer. The formulations ranged from 596 nm to 1365 nm in size (Table 2).

Table 2 Particle size and size distribution of various batches of phytosomes

Formulation Code	Particle Size (nm)
TP 1	596
TP 2	1248
TP 3	638
TP 4	1139
TP 5	1008
TP 6	1094
TP 7	943
TP 8	1365
TP 9	731

It was evident from the results of the particle size that the amount of lipid and extract had a significant effect on the particle size of the phytosome. The phytosomes prepared with lower lipid were found to be of higher sizes whereas those with higher concentration of the lipid were small in size. On the other hand, increasing the amount of extract was found to increase the particle size of the phytosomes. The influence of lipid and extract concentration on particle size was studied using design expert 7.0.0 trial version. The ANOVA for the selected factorial model suggested the equation to predict the particle size as under.

Particle size = 973+197.11*A1-30.56*A2-226.22*B1-35.89B2

The model had a predicted R-squared value of 0.9651 with a standard deviation of 71.49.

Table 3 Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Studentized Residual	Fitted Value / DFFITS	Run Order
1	1008	944.44	63.56	0.556	1.550	1.732	5
2	638	716	-78-78	0.556	-2.542	*-2.84	3
3	596	580.78	15.22	0.556	0.280	0.313	1
4	1139	1134	4.22	0.556	0.077	0.086	4
5	943	907.11	35.89	0.556	0.704	0.787	7
6	731	771.11	-40.11	0.556	-0.803	-0.898	9
7	1365	1432.78	-67.78	0.556	-1.751	-1.958	8
8	1248	1205.11	42.89	0.556	0.873	0.976	2
9	1094	1069.11	24.89	0.556	0.468	0.524	6

*Exceeds Limit

The plot of predicted particle size vs. the actual particle size is shown in Figure 2.

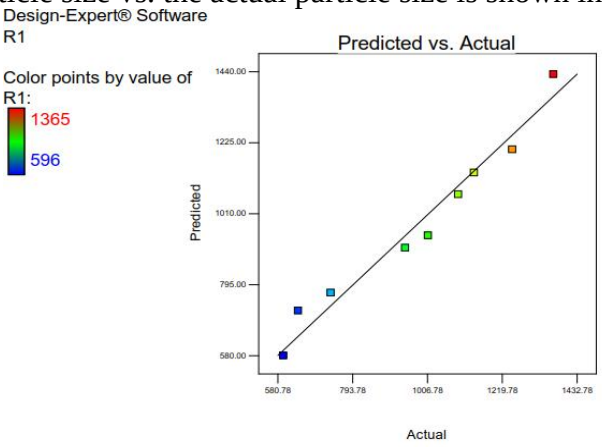

Figure 2 Predicted vs. Actual

The solutions for numerical optimization of lipid and extract concentrations were generated using the model. It led to 9 possible combinations of which the combination with the highest desirability was graphically evaluated.

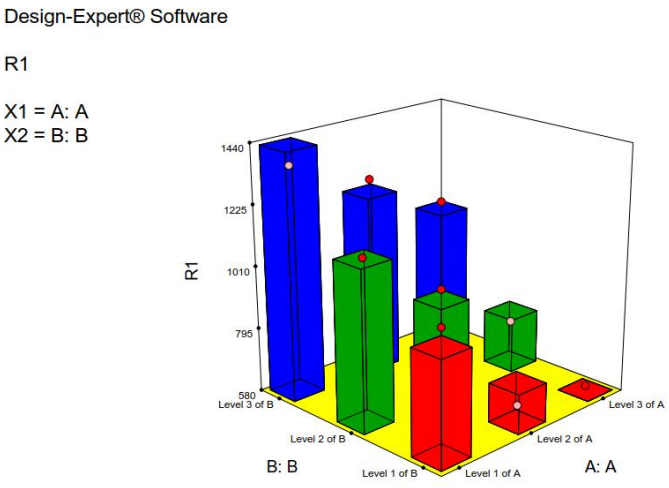

Figure 3 Particles size using the selected solution for optimization

The point prediction suggested level 3 (0.3 g) of lipid concentration and level 1 (0.1 g) of extract concentration to present the lowest particle size with the minimum residual in the predictive ability.

Evaluation of phytosomes

Surface morphology (visualization)

The selected phytosomes with lowest particle size (**TP1**) were analyzed under and electron microscope. The particles were visible as rigid, almost spherical vesicles in SEM image. The surface of the phytosome vesicles was found to be regular and smooth (Figure 4).

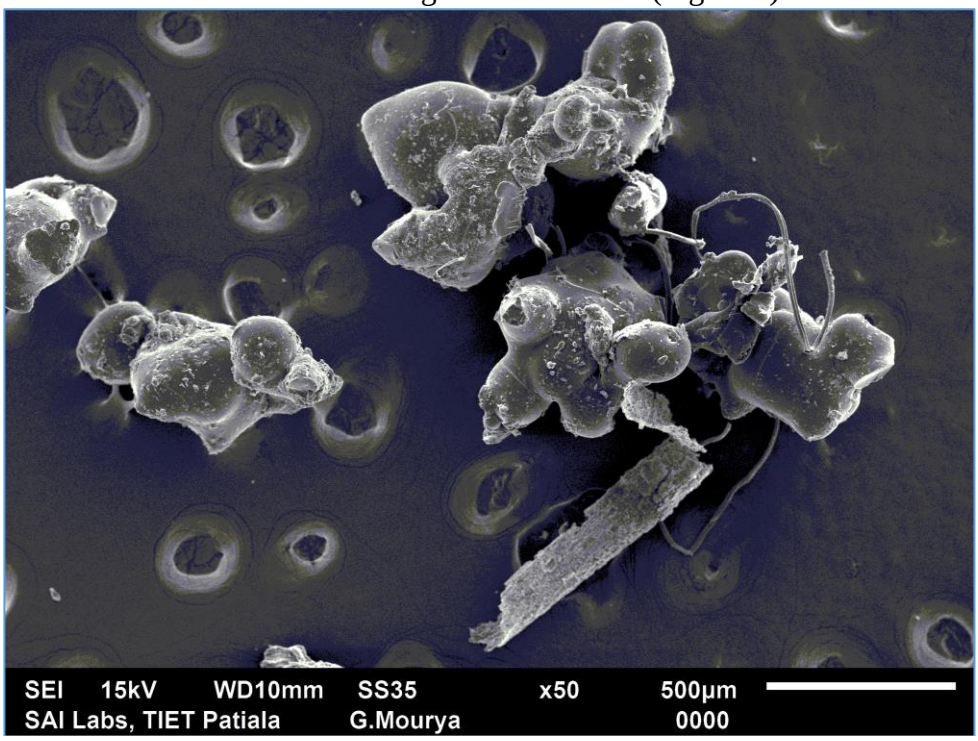

Figure 4 SEM Image of TP 1

Differential Scanning Calorimetry

The thermogram of soya lecithin gives distinct peak at 57°C indicating melting (Figure 5a). Sharp endothermal peak was found in the thermogram of the phytosome at 257°C (Figure 5b).

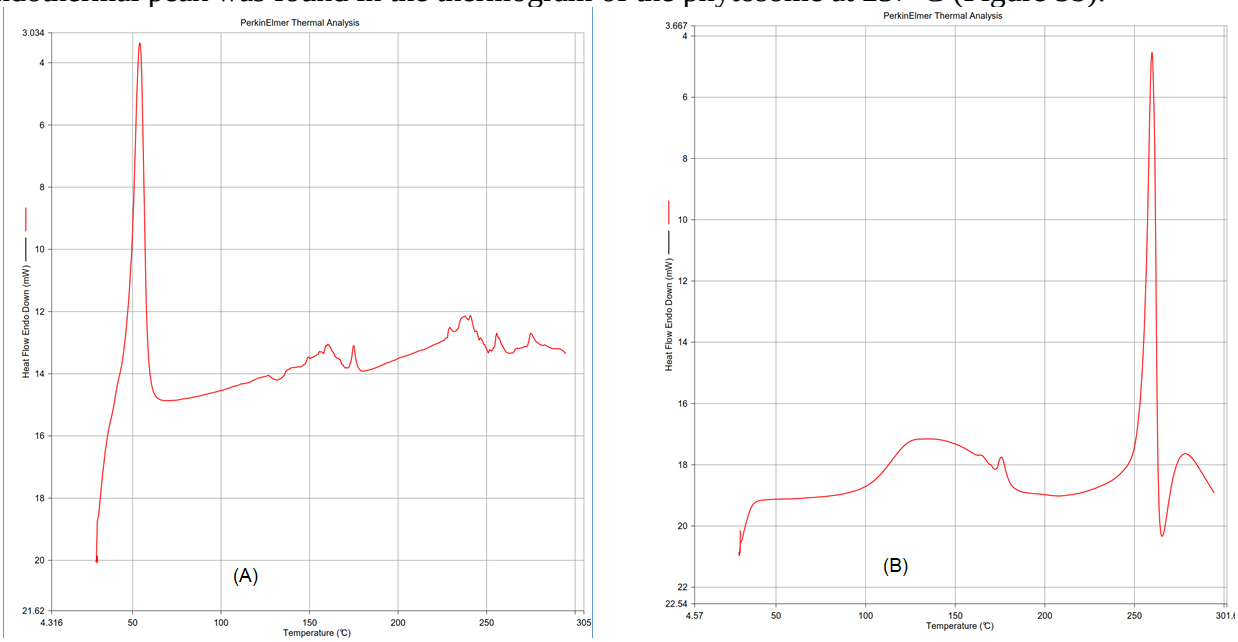

Figure 5 (A) DSC of Lecithin (B) Phytosome T1

From the DSC it can be concluded that a stable formulation is formed by some molecular interaction that can be either van der waals forces or hydrogen bonding between extract and phospholipids that distributed the extract molecularly into phospholipid.

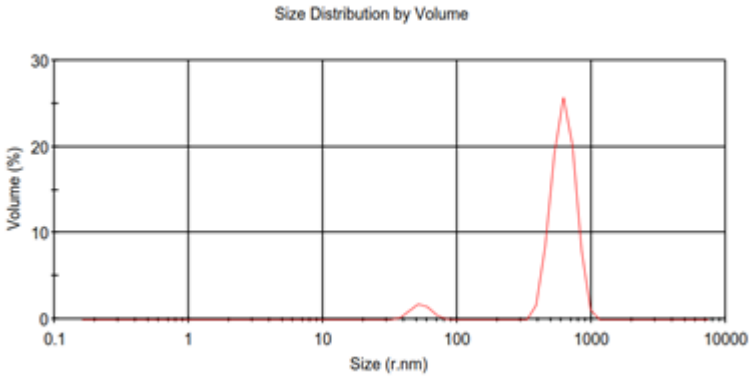

Figure 6 Particle size of TP 1

Antioxidant action of phytosomes

DPPH scavenging assay was used to analyze the effect of extract encapsulated in phytosome with reference to the extract directly (Table 4). The IC50 value of DPPH inhibition was calculated by plotting the curve of inhibition vs. concentration and calculating the regression equation (Figure 7). It was observed that the IC_{50} exhibited by the extract as well as the IC_{50} exhibited by the phytosome loaded with extract was almost similar (116.48 µg/mL vs. 113.96 µg/mL).

The formulation of the extract as phytosome is supposed to improve the half life as well as bioavailability of the extract and also prolong its duration of action by reducing its elimination. Considering the similar IC_{50} values of both, it is evident that phytosomes improve the antioxidant potential of the extract.

Table 4 DPPH radical scavenging assay of the phytosome and extract

	% DPPH Inhibition				
Dose (µg/mL)	**50**	**100**	**150**	**200**	**250**
Extract	27.2	44.3	63.9	82.7	93.6
Phytosome	24.8	41.9	67.4	80.5	96.2

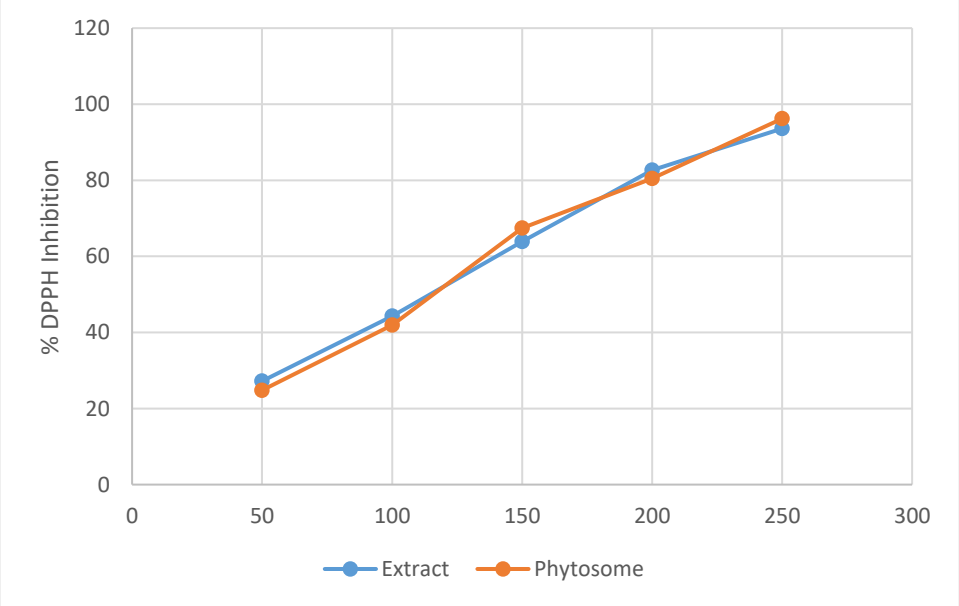

Figure 7 Percent DPPH Inhibition curve

Conclusion

The study presented in this thesis reveals the excellent potential of phytosome based drug delivery system for maintaining the antioxidant activity of plant extract while improving the acceptability of the formulation. We can conclude that phytosome based formulation could be a valuable approach to improve the therapeutic efficacy, to reduce dose and improvement in dosage regimen for plant extracts and phytoconstituents.

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