



INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X

Impact factor: 4.295

(Volume 5, Issue 5)

Available online at: www.ijariit.com

Formulation and evaluation of Adulsa capsules Phytosomes

Nahida Shaikh

nahidaskh@gmail.com

Principal K. M. Kundnani College of Pharmacy,
Mumbai, Maharashtra

Dr. Swati Patil

drswatispatil@gmail.com

Principal K. M. Kundnani College of Pharmacy,
Mumbai, Maharashtra

ABSTRACT

The major problem associated with the herbal drug is poor bioavailability so as to improve the bioavailability of herbal novel drug delivery system known as phytosome is used. By applying this novel approach bioavailability is increased. The objective of the current project is to develop a formulation with a systematic approach to yield better products in terms of stability, acceptability and therapeutic value owing to its greater bioavailability origin. Different phytosomal complexes of vasaka leaf extract containing a molar ratio of 1:1,1:2,1:3,1:4,2:1,2:2 using methanolic extract, soya lecithin and cholesterol were prepared by solvent injection technique. *Adhatoda vasica* phytosomes were characterized by particle size, zeta potential, entrapment efficiency, Fourier transform infrared spectroscopy and in vitro drug release. The average particle size and zeta potential of optimized formulation were found to be 136.7nm and -22.7 and entrapment efficiency was found to be 71%. In-vitro % drug release of capsules of *Adhatoda vasica* was found to be 85%.

Keywords— *Adhatoda Vasica* Extract, *Adhatoda Vasica* Phytosomes, Bioavailability, Lipid

1. INTRODUCTION

Nature always stands as a golden mark exemplify the outstanding phenomenon of symbiosis and is still mankind's greatest chemist. People are now showing interest in natural medicines then synthetic once, primarily because of a high degree of adverse side effects caused by the latter. The demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics, etc. is increasing day by day. The plant *Adhatoda vasica* commonly known as vasaka, family Acanthaceae, is used traditionally for treating cold, cough, whooping cough, chronic bronchitis, asthma and also acts as a sedative, expectorant, antispasmodic. *Adhatoda vasica* is an important source of phytoconstituents like quinazoline alkaloid vasicine, vasicinone, vasicinol, vasicinine, and vasicoline. The alkaloids vasicine and vasicinone show bronchodilatory action [1]. Phytosome is a newly introduced patented technology developed to incorporate the standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called phytosomes. Phytosomes provide better absorption and bioavailability than conventional herbal extracts. When a stoichiometric amount of the phospholipid was made to react with purified herbal extract in an aprotic solvent, phytosomes were formed. The objective of this study is to formulate and evaluate capsules of *Adhatoda vasica* [2].

2. MATERIALS AND METHOD

Lipid P-100 was obtained as a gift sample from lipoid, Germany, *Adhatoda vasica* methanolic extract Cholesterol, Phosphate buffer 7.4, ethanol, distilled water, Methanol (HPLC grade), Acetonitrile (HPLC grade), HPLC Water.

2.1 Method

2.1.1 Collection of leaves: Fresh leaves of Vasaka were collected from the medicinal plant Garden of Prin. K. M. Kundnani College of pharmacy colaba (Mumbai) during the month of February 2018.

2.1.2 Authentication of leaves: Authentication of fresh vasaka leaves along with the flower was carried out at Saint Xavier's College, Mumbai. The sample number P.D.580 matched with the standard herbarium specimen and its identity was confirmed to be *Adhatoda vasica* Linn. Family: A canthaceae.

2.1.3 Processing of leaves: The fresh leaves were washed with water and wiped with a clean cloth to remove dirt, dust, and debris. The leaves were air dried at room temperature for 10 days. The dried leaves were then grinded to powder.

2.1.4 Extraction of leaves ^[3]: *Adhatoda vasica* dried leaves were powdered and were subjected to defatting with petroleum ether using the Soxhlet apparatus as solvent for 6 to 8 hours so as to remove fatty material and chlorophyll. After leaves were defatted, the remaining marc was extracted using methanol until the siphon tube showed colourless liquid. The combined extract obtained was evaporated to dryness using an electric water bath to yield the crude extract. The methanolic extract was then subjected to phytochemical evaluation to determine the presence of various phytoconstituents viz. flavonoids, terpenoids, alkaloids, glycosides, saponins, tannins, phenols.

2.1.5 Phytochemical screening ^[4]: Preliminary phytochemical investigations were carried out methanolic extract to detect the presence of phytochemicals using standard method

2.1.6 TLC analysis of methanolic extract ^[5]: Thin layer chromatography was carried out on a precoated silica gel 60 F254 plates. The mobile phase was optimised using different solvent systems.

2.2 Determination of maximum wavelength of absorption

10mg of *Adhatoda vasica* methanolic extract was dissolved in 10ml of methanol, 0.1NHCL and phosphate buffer 7.4 to get a concentration of 1000µg/ml. From this 1000µg/ml stock solution 1.6ml was taken and diluted with methanol, 0.1N HCL and phosphate buffer 7.4 seperately...The prepared solution was scanned in the range of 200-800 on a thermoscientific 300 UV-vis spectrophotometer. Absorbance maxima were found to be 281nm.

2.3 Preparation of standard calibration curve

The stock solution of 1000µg/ml was prepared by dissolving 10 mg of extract in 10 ml of methanol. From this stock solution 0.4ml, 0.8ml, 1.2ml, 1.6ml, 2.0ml, 2.4ml were withdrawn and diluted to 10ml with methanol, 0.1N HCL and phosphate buffer 7.4. Different stock solution of 40µg/ml, 80µg/ml, 120µg/ml, 160µg/ml, 200µg/ml, 240µg/ml, were made from this stock solution of 1000µg/ml. The standard solution with concentration ranging from 40-240µg/ml was obtained. The absorbance of this solution was observed at 281nm.

3. HPLC ANALYSIS OF METHANOLIC EXTRACT OF ADHATODA VASICA

HPLC method development was carried out using methanol: water (2:3) as a mobile phase. A stainless steel column of 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica was used. The chromatogram of extract was compared with the standard vasicine chromatogram mentioned in literature survey.

3.1 Material and Reagents

Methanol (HPLC grade), Acetonitrile (HPLC grade), HPLC Water, Methanolic extract of leaves.

3.2 Preparation of Sample Solution

10mg of the extract was dissolved in 10ml of methanol to prepare of stock solution of 1000µg/ml/.from this stock solution 1ml was removed and further diluted with 10ml of methanol to prepare a stock solution of 100 µg/ml. The final prepared stock solution of 100µg/ml was sonicated on water bath for 30 min. After sonication it was filtered through Whatmann filter paper and was used for the chromatography. The same procedure was followed for marketed formulation and phytosomal formulation.

4. PRELIMINARY-SCREENING

A drug excipient compatibility study was carried out by FTIR spectra.

5. ADHATODA VASICA PHYTOSOME FORMULATION DEVELOPMENT

5.1 Phytosome of *Adhatoda vasica* was prepared by the solvent injection technique ^[6].

- The required amount of methanolic extract of vasaka, lipids i.e. Lipoid P-100 and Cholesterol in 1:2 were dissolved in 5 ml Ethanol by ultrasonication.
- In another beaker, 15ml Phosphate buffer 7.4 was taken and kept for stirring at 1200 rpm using magnetic stirrer (Remi) at room temperature. To the aqueous phase, the lipid phase was added by injection at one jet.
- The mixture was stirred continuously for 2 hours to allow solvent evaporation and to obtain uniform vesicular dispersion.
- The dispersion was then homogenised in a high-speed homogenizer to form small uniform size vesicles.

6. CHARACTERISATION OF PHYTOSOME FORMULATION.

6.1 Particle size and Zeta potential

Particle size and zeta potential measurements were carried out using HORIBA Scientific SZ-100. Zeta potential is the most important parameter for physical stability of phytosomes. The higher the electrostatic repulsion between the particles the greater is the stability ^[2]

6.2 Entrapment Efficiency

The entrapment efficiency of vasaka phytosome vesicles was determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge (REMI ultra-centrifugation) at 10,000 rpm for 90 minutes at a temperature maintained at 4°C. The concentration of the free drug as the supernatant was determined by measuring absorbance at 281nm using a UV-Visible spectrophotometer ^[7]. The percentage drug entrapment was calculated by using the formula:

$$\text{Entrapment Efficiency} = \frac{\text{Weight of total drug} - \text{Weight of final drug}}{\text{Weight of total drug}} \times 100$$

6.3 Stability studies

The stability of vasaka phytosomes was carried out as per ICH guidelines. The optimized formulations were stored at different temperature ranges $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $25^{\circ}\text{C} \pm 20^{\circ}\text{C}$ for a period of 3 months and studied for drug entrapment [8]

6.4 In-vitro release study

The release study was carried in 250ml beaker containing 200 ml of diffusion medium phosphate buffer solution pH 7.4. The dialysis membrane previously soaked overnight in the diffusion medium was used for the study. The dialysis membrane was used as a barrier to isolate between donor and receptor phase. Dialysis membrane measuring 10cm was taken and one end of membrane was tied with the help of thread. Phytosome formulation 2ml was filled from another end into the dialysis membrane and tie with thread. The dialysis membrane containing the phytosome was suspended into the medium. Beaker assembled on a magnetic stirrer and equilibrated at 37°C . Content of beaker was kept to stirred at 100rpm and aliquots were withdrawn at 0, 5, 15, 30, 45, 1, 2, 3, 4, 5, 6, 8, 24 hour and replaced by 3 ml volume of phosphate buffer pH 7.4 to maintain the sink condition [24] These samples were analyzed by UV Spectrophotometer at $\lambda_{\text{max}} 281\text{nm}$ [9]

6.5 In vitro dissolution testing

Dissolution profiles of capsule formulation containing *Adhatoda vasica* phytosome, pure extract, and marketed formulation were determined according to USP Basket method (USP XXI/XXII Model TQT-06, Electrolab, India) using 900 ml of phosphate buffer (pH 7.4) as dissolution media (maintained at $37 \pm 0.5^{\circ}\text{C}$) at 100 rpm. 5 ml aliquots of of dissolution medium collected at different intervals of time (0, 15, 30, 45 and 60 min) and were replenished with equal volume of fresh media after each sampling so as to maintain the constant volume of the medium. The samples were analyzed at 281nm using UV-visible spectrophotometer [10]

7. RESULTS AND DISCUSSION

7.1 Extraction Yield

Table 1: Percent yield of an extract of *Adhatoda vasica*

Name of extract	%yield
<i>Adhatoda vasica</i> methanolic extract	10% w/w

7.2 TLC

Mobile phase: ethylacetate:methanol:strong ammonia (8:0.5:0.2)

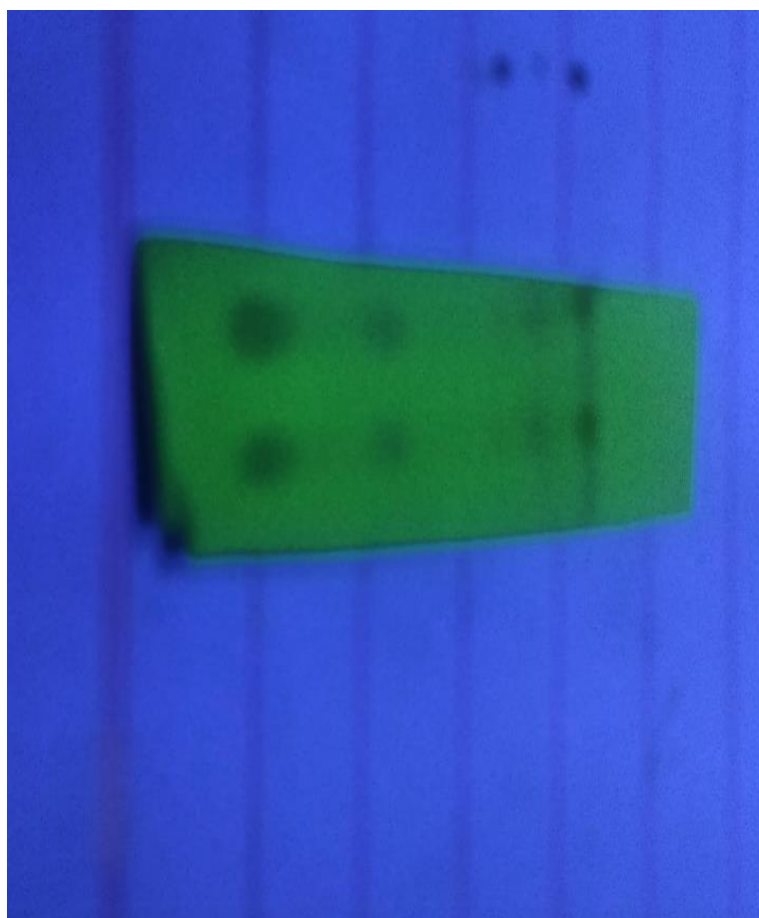


Fig. 1: Final TLC of methanolic extract

7.3 Phytochemical Evaluation

The phytochemical screening of the methanolic extract showed the presence of alkaloids, carbohydrate tannins, sterols, phenols.

7.4 Determination of Absorbance Maxima

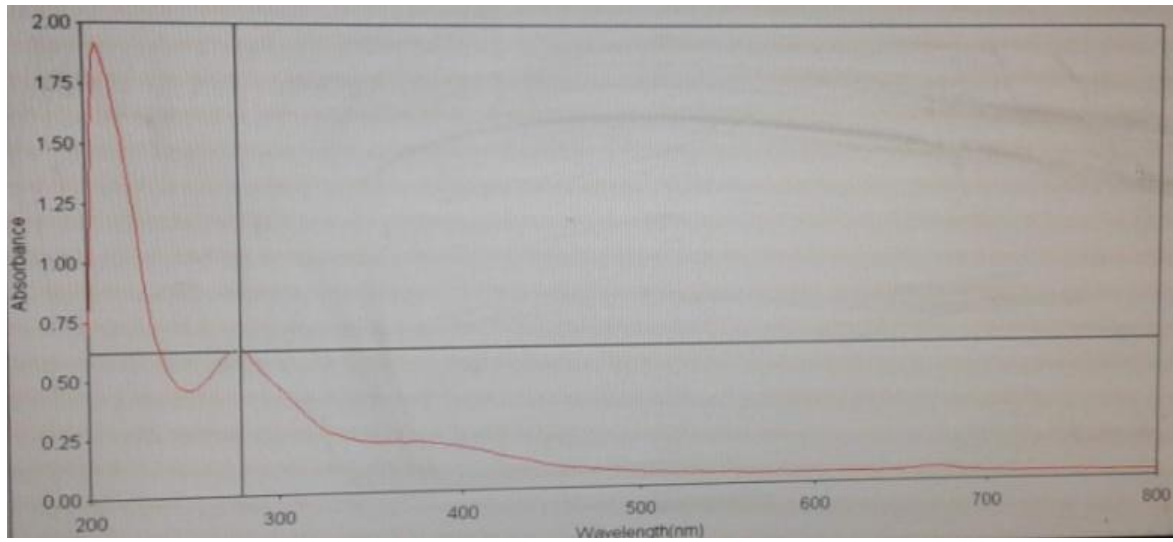


Fig. 2: UV-Vis spectrum of *Adhatoda vasica* extract in phosphate buffer 7.4

7.5 Calibration Curve of Adhatoda Vasica Extract

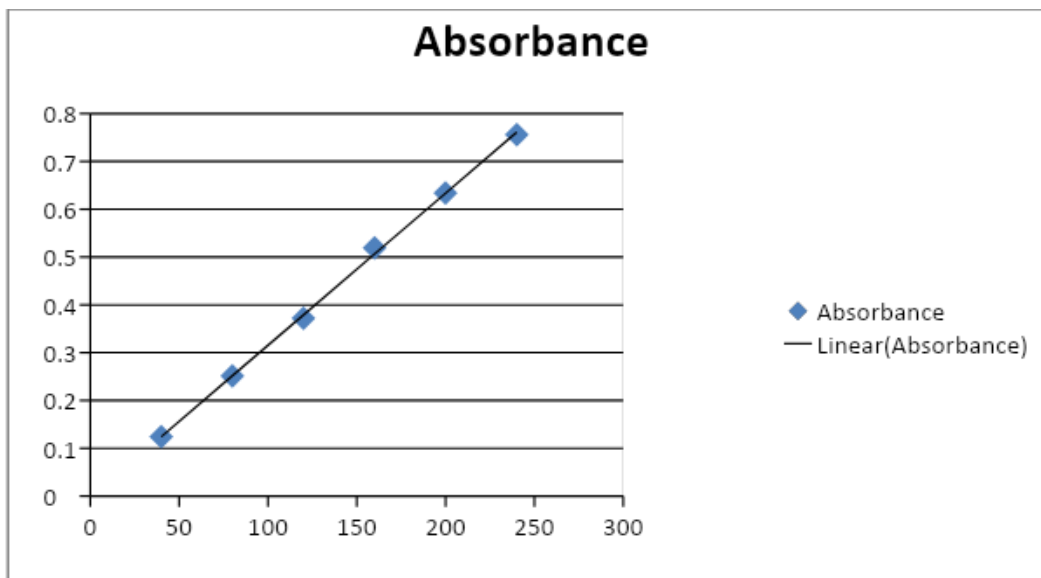


Fig. 3: Calibration curve of *Adhatoda vasica* methanolic extract in phosphate buffer 7.4

7.6 HPLC analysis of Methanolic extract of Adhatoda Vasica

HPLC method development was carried out using methanol: water (2:3) as a mobile phase. A stainless-steel column of 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica was used.

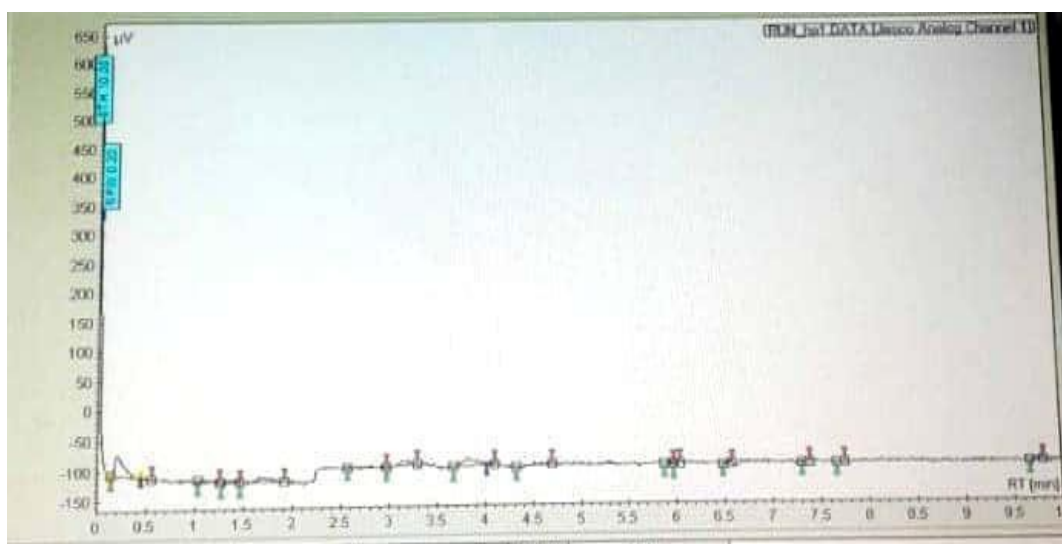


Fig. 4: Blank chromatogram in the final mobile phase

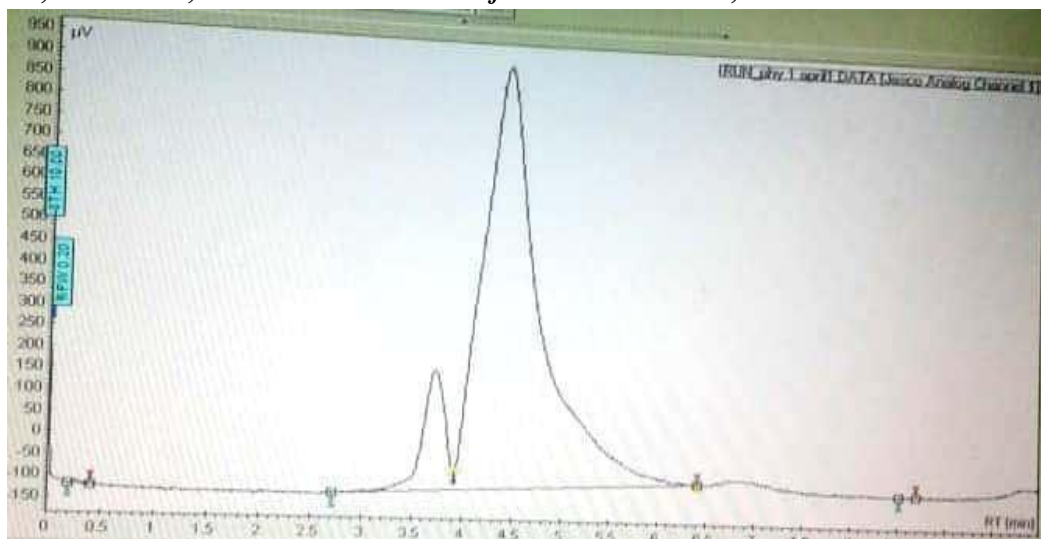


Fig. 5: HPLC Chromatogram of *Adhatoda vasica* pure extract

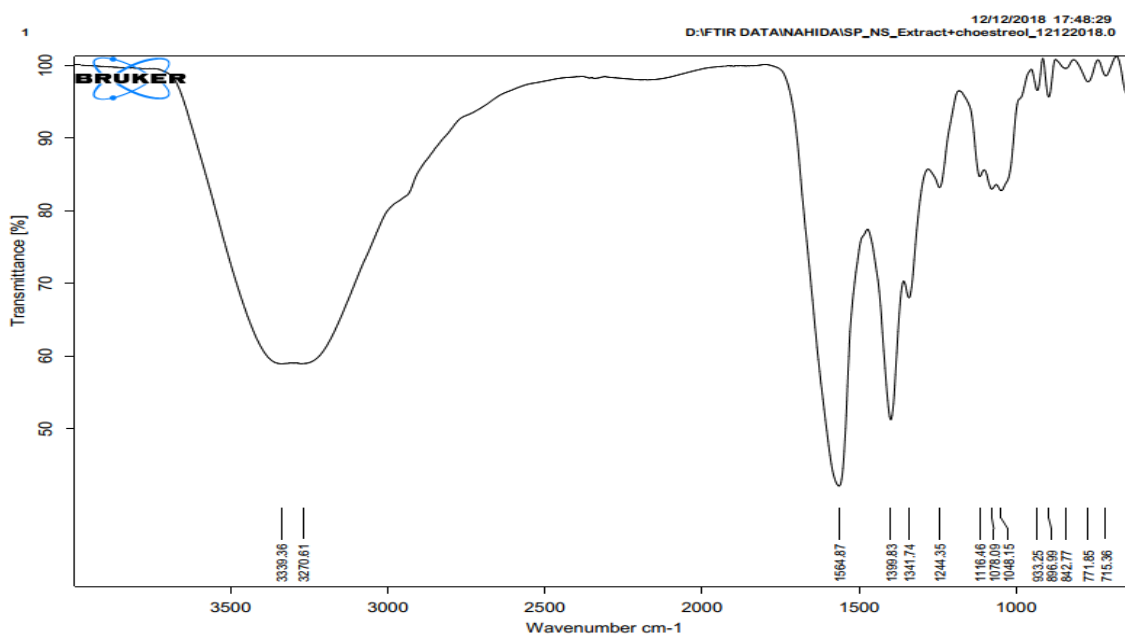


Fig. 6: FTIR graph of *Adhatoda vasica* pure extract and cholesterol

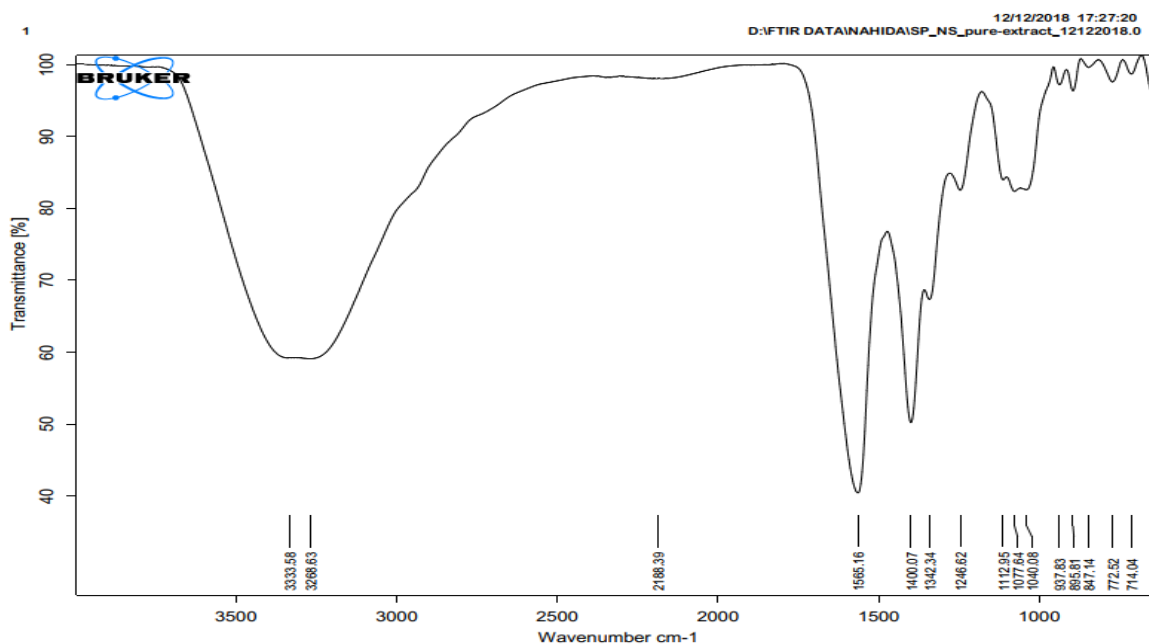


Fig. 7: FTIR graph of *Adhatoda vasica* pure extract

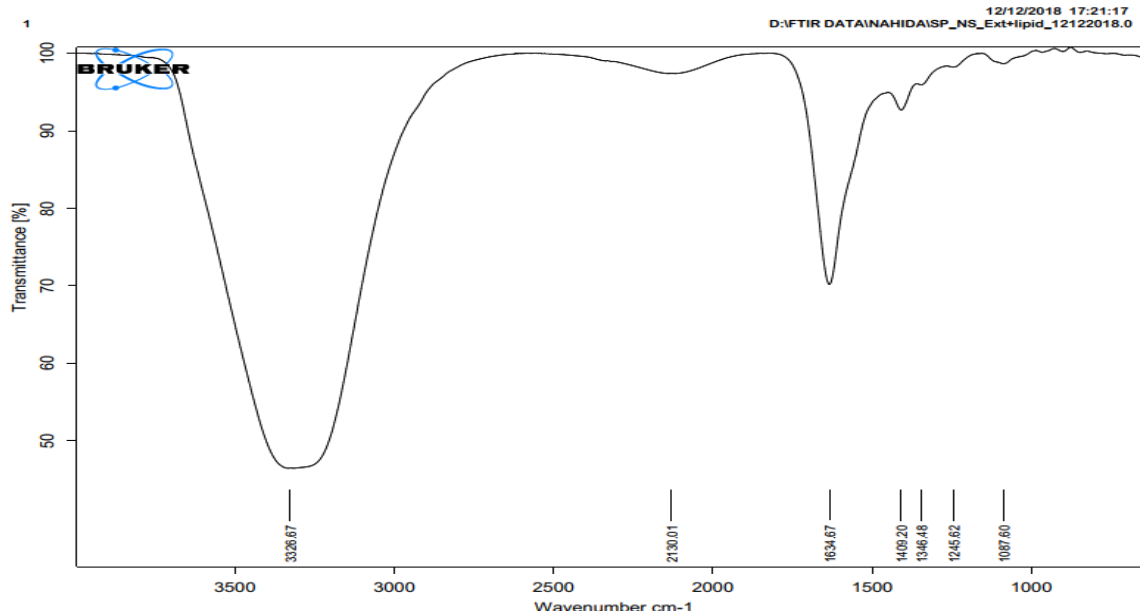


Fig. 8: FTIR graph of *Adhatoda vasica* pure extract

Table 2: Optimization of phytosome of *Adhatoda Vasica*

Formulation code	Extract (mg)	Lipid-100 (mg)	Cholestrol (mg)	PB 7.4 (ml)	Ethanol (ml)	Ratio
F1	20mg	20mg	2mg	15ml	5ml	1:1
F2	20mg	40mg	3mg	15ml	5ml	1:2
F3	20mg	60mg	6mg	15ml	5ml	1:3
F4	20mg	80mg	7mg	15ml	5ml	1:4
F5	40mg	20mg	8mg	15ml	5ml	2:1
F6	40mg	40mg	9mg	15ml	5m	2:2

8. CHARACTERISTIC OF PHYTOSOMES FORMULATION

8.1 Measurement of particle size

The particle size of the optimized F2 batch was found to 136.7 nm and polydispersibility index was found to be 0.367. The particle size of phytosomes is extremely important as it directly affects the stability and bioavailability of phytoconstituent encapsulated systems. Smaller particles possess a large surface area and have faster release as well as higher stability [11]

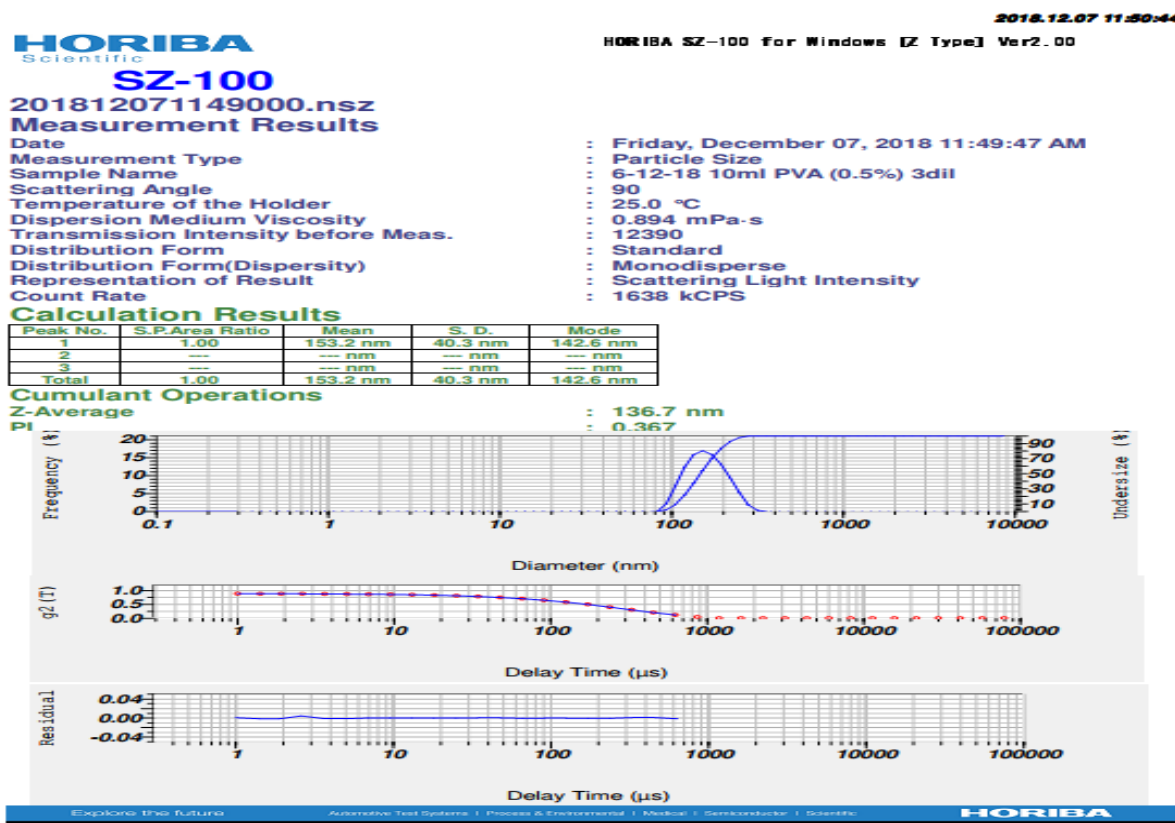


Fig 9: Particle size distribution of *Adhatoda vasica* phytosome

8.2 Measurement of zeta potentials

The zeta potential of a particle is the overall charge that a particle acquires in a particular medium. It is a physical property which is exhibited by any particle in suspension. Zeta potential is a very good index of the interaction magnitude between colloidal particles. Evaluation of zeta potential help to predict the stability of colloidal systems [12]

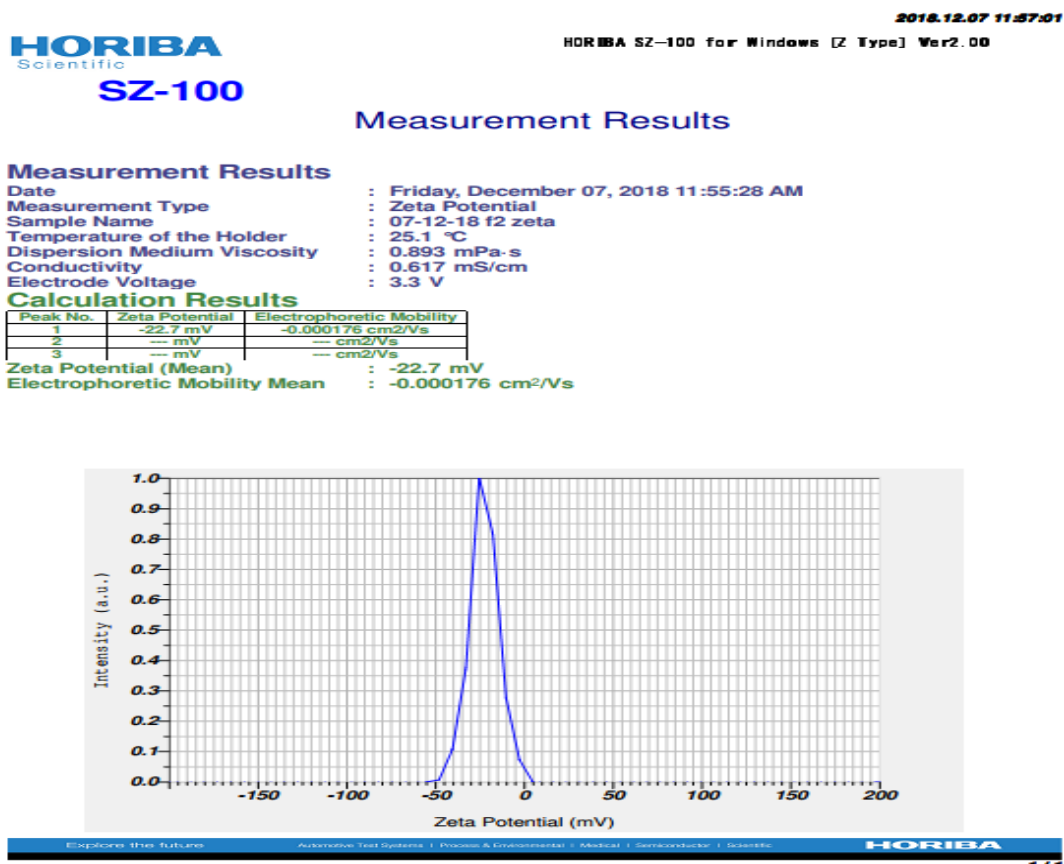


Fig 10: Zeta potential of *Adhatoda vasica* phytosome

8.3 Entrapment Efficiency

The entrapment efficiency of phytosome was determined by centrifugation method .F2 formulation showed maximum entrapment efficiency of 71%.

$$\text{Entrapment Efficiency} = \frac{\text{Weight of total drug} - \text{Weight of final drug}}{\text{Weight of the total drug}} \times 100$$

Table 3: Entrapment efficiency of phytosome formulation

Extract: lipid	Ratio	Formulation code	Entrapment efficiency (%)
20:20	1:1	F1	70
20:40	1:2	F2	71
20:60	1:3	F3	67
20:80	1:4	F3	61
40:20	2:1	F4	54
40:40	2:2	F5	52

8.4 Stability studies

The stability of vasaka phytosomes was carried out as per ICH guidelines. The optimized formulations were stored at different temperature ranges 4°C ± 2°C, 25°C ± 20°C for a period of 3 months and studied for drug entrapment.

Table 4: Stability study of final (F2) phytosome

S no.	Temperature	Time	Particle size(nm)	Entrapment Efficiency
1	4°C ± 2°C	Initial	136.7	71
		1 month	142	68
		2 months	148	63
		3 months	151	59
2	25°C ± 2°C	Initial	136.7	71
		1 month	144	69
		2 months	156	65
		3 months	150	60

8.5 In-vitro release study

In vitro release study was carried out using dialysis technique. F2 formulation showed highest release due to its lowest particle size. F2 formulation showed release of 75% whereas a pure extract of *Adhatoda vasica* extract showed a release of 48.5% and marketed formulation showed a release of 42% at the end of 12 hr study.

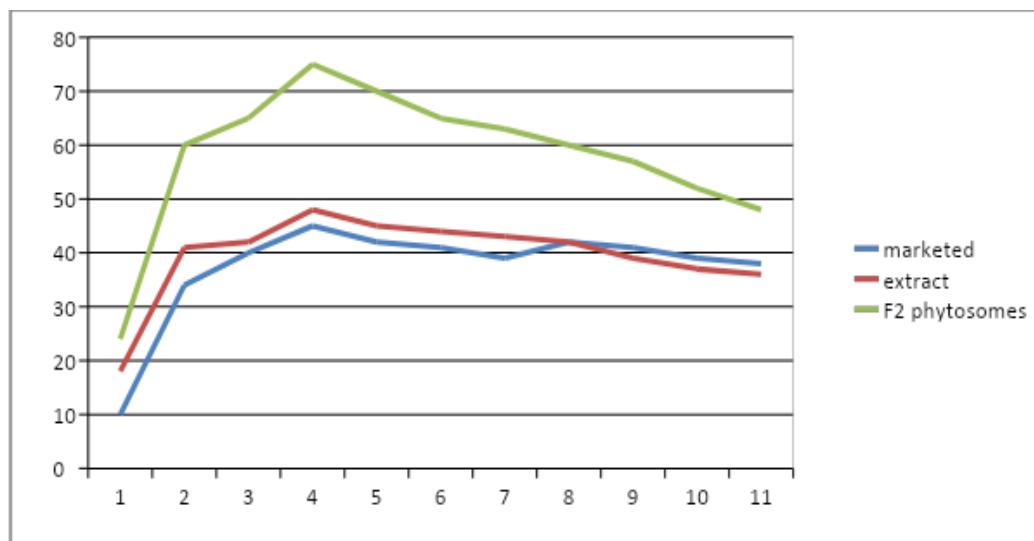


Fig. 11: In-vitro release from phytosome formulation, marketed and *Adhatoda vasica* methanolic extract

8.6 In-Vitro Dissolution Study

Adhatoda vasica phytosome capsule showed the release of 85% whereas marketed formulation showed release of 76.39% and that of pure extract showed release of 80%.

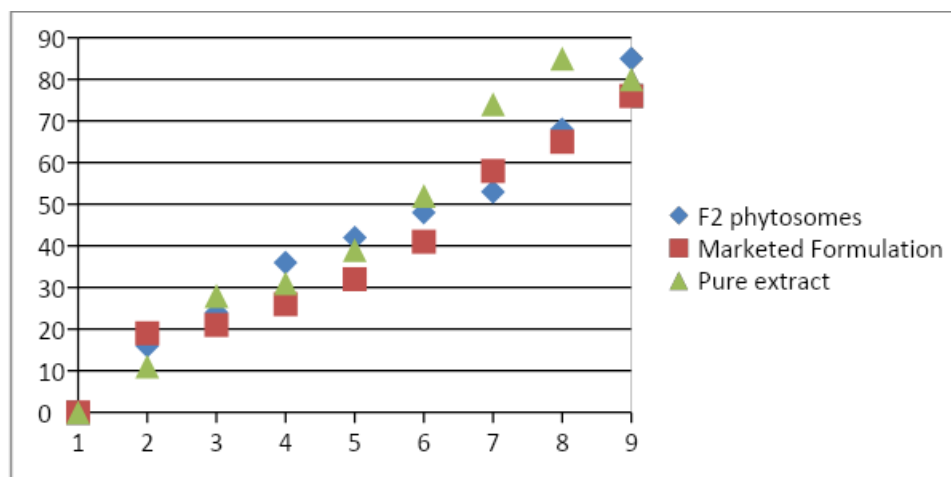


Fig. 12: *Adhatoda vasica* Phytosome capsule release compared with the marketed formulation and pure extract.

9. CONCLUSION

Adhatoda vasica commonly known as vasaka is the most powerful herb for the treatment of the respiratory problem. The phytosome technology help in improving bioavailability of herbal drugs. The phytosome prepared by novel technique showed better release as compared to the conventional marketed formulation. Applying the modern form of technology to yield the herb formulation can be a new paradigm in herbal therapeutics. The objective of current project is to develop a formulation with systematic approach to yield better product in terms of stability, acceptability and therapeutic value owing to its greater bioavailability origin

10. REFERENCES

- [1] Khursheed, A.; Devender, P.; Ansari, S. Phytochemical and Pharmacological Investigations on *Adhatoda Zeylanica* (Medic.): A Review. *Pharmacogn. J.* **2013**, 2 (12), 513–519. [https://doi.org/10.1016/s0975-3575\(10\)80041-0](https://doi.org/10.1016/s0975-3575(10)80041-0).
- [2] 2-B, K.; Pingali, S.; Srinivas, P. Formulation and Evaluation of Capsule of *Aswagandha* Phytosomes. *Int. R. Sci. Rev. Res.* **2014**, 29(2), 138-142.
- [3] Dhase, A. S.; Saboo, S. S. Preparation, and Evaluation of Phytosomes Containing Methanolic Extract of Leaves of *Aegle Marmelos* (Bael). *Int. J. PharmTech Res.* **2015**, 8 (6), 231–240.
- [4] Singh, S.; Hussain, A.; Singh D. Phytochemical Screening and Determination of Quinazoline Alkaloid In *Adhatoda vasica*. *Int. J. Pharm. Sci. Rev. Res.* 2012, 14(2), 115-118.
- [5] Sajeeb, B. K.; Kumar, U.; Rouf, A. S. S.; Uddin, M. A.; Bachar, S. C. Evaluation of *Justicia Adhatoda* L. Syn. *Adhatoda Vasica* Nees Extract by Major Metabolite Analysis. *Indian J. Tradit. Knowl.* **2015**, 14 (4), 637–642.

- [6] Lankalapalli, S.; Vinai Kumar Tenneti, V, S.; Adama, R; Malleswara, N.;Beeraka, R.Preparation and Evaluation of Liposome Formulations for Poorly Soluble Drug Itraconazole by Complexation. *Der Pharm.Lett.* **2015**, 7(9), 12-29.
- [7] Sahu, A. R.; Bothara, S. B. Formulation, and Evaluation of Phytosome Drug Delivery System of Boswellia Serrata Extract. *Int. J. Res. Med.* 2015, 4 (2), 94–99.
- [8] Latha, S. M.; Devi, N.G.;Anusha,N.;Sameera,M.;Silpa,N.;Lakshmi,N.M.Formulation and Comparative Evulation of Aceclofenac Tablets by Two Granulation Methods. *Int.J.Pharm.Clin. Res.* **2016**.8(7), 649-654.
- [9] Lala, R.R. Artemether Loaded Liposomes for enhanced Intestinal Permeability: Formulation Development and Evaluation. *World J. Pharm.Pharm.Sci.* **2017**,6(4), 1596-1608.
- [10] Lo, L.; Lu, X.; Lloyd, D. Dissolution Testing of a Controlled Release Capsule Formulation: Challenges and Solutions Using a Semi-Automated Dissolution System. *Dissolution Technol.* **2013**, 20 (2), 6–13.
- [11] Rasaie, S.; Ghanbarzadeh, S.; Mohammadi, M.; Hamishehkar, H. Nano Phytosomes of Quercetin : A Promising Formulation for Fortification of Food Products with Antioxidants Nano Phytosomes of Quercetin : A Promising Formulation for Fortification of Food Products with Antioxidants. *Pharm, Sci.*2014, 20, 96-101.
- [12] Laouini, A.; Jaafar-Maalej, C.; Limayem-Blouza, I.; Sfar, S.; Charcosset, C.; Fessi, H. Preparation, Characterization and Applications of Liposomes: State of the Art. *J. Colloid Sci. Biotechnol.* **2012**, 1 (2), 147–168. <https://doi.org/10.1166/jcsb.2012.1020>.