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Management of human cervical cancer using polyherbal phytosome: An *in-vitro* analysis

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Abstract
Herbal drugs are widely accepted in the field of medicine for the treatment of diseases. However, the procurement and processing of herbals are not only difficult but quite elaborate. From collection, preparation and evaluation of the extract, which has to comply with the standards results in receiving a small amount of plant actives though huge labour and solvents are involved. In addition, many phytoconstituents don't attain the required dose in the targeted organ or tissue due to poor lipid solubility or larger molecular size resulting in reduced absorption and bioavailability thus requiring more concentration of drug in the dosage form. Therefore, novel drug delivery systems are employed to enhance their lipid solubility to diffuse through lipid- rich biological membranes. One of this technology is Phytosome, a new technique which incorporates extracts or phytoconstituents whose lipid solubility has to be enhanced to pass through biological layers with phospholipids leading to increased absorption and bioavailability so that its action is improved and therefore involving a lesser amount of extract concentrate.
Cassia tora and Panchvalkal (an Ayurvedic preparation) are traditionally known for their medicinal properties which have been reviewed and examined by various studies. The modern medicine system though has made progress in treating different cancers, it is not free from side effects. The rationale of the research project was to combine them and formulate them into Phytosome formulation. The formulation thus made was optimization for maximum entrapment efficiency. Phytosome was produced using solvent injection method using phosphatidylcholine and cholesterol. Evaluation parameters like %Entrapment efficiency, particle size, Zeta, potential was performed. In-vitro diffusion study of phytosome exhibited 74.86% of cumulative drug release upto 24hrs. In-vitro cytotoxicity of phytosome complex on Human cervical cancer cell line (HeLa) was evaluated by using Cisplatin as standard drug. A novel drug delivery system that has been formulated thus improves the efficacy of the formulation which could be useful to treat any malignant transformations although sustaining properties of phytoconstituents.

Keywords: *Cassia tora*, panchvalkal, phytosome, anticancer, cervical cancer, polyherbal

Introduction
The earth is gifted with bounteous herbs and plants. Plants have been an exemplary model for the discovery of medicine. Natural products have always been propitious for human health care. Plants have been a tremendous source of medicinally important compounds since the origin of mankind. Herbal plants have been the fundamentals of the traditional system of medicine, one such is Ayurveda which uses the concept of Polyherbalism. Various phytochemicals present in polyherbal formulations can display potent anti-cancer properties as they contain a combination of compatible herbs which can intensify its therapeutic effect to a desired extent [1].
Cassia tora Linn. which is also known as *Senna tora* belonging to the family Leguminosae, is a ubiquitous plant that propagates as a weed in moist, damp soil found in tropical parts of the Indian subcontinent [2, 3]. Several literature studies have reported the presence of phytochemicals such as emodin, chrysophanol, aloe-emodin, physcion, obtusin, obtusifolin chryso-obtusifolin-2-O- β -D-glucoside, β-sitosterol, kaempferol-2-diglucoside (Flavonol glycoside), ononitol monohydrate from various parts of the plant contributing to several pharmacological activities like laxative, antiperiodic, anthelmintic, antimicrobial, antifungal, anticancer, anti- psoriatic, anti-parkinsonian, anti-nociceptive, anti-ulcer, anti-diabetic, antioxidant, anti-inflammatory, antifertility, anti-arthritic, anti-asthmatic, anti-depressant, anti-amnesic, anti-epileptic, ophthalmic, liver tonic, cardiotonic, and expectorant [4] [5].
Panchvalkal is a mixture of barks of Ficus plant species i.e. [6] (Table 1)

Table 1: Constituents of panchvalkal

Botanical Name	Classical name	Family name	Proportion (in parts)
Ficus bengalensis Linn.	Vata chhal	Moraceae	1
Ficus religiosa Linn.	Ashwatha chhal	Moraceae	1
Ficus racemosa	Udumbara chhal	Moraceae	1
Ficus lacor	Plaksha chhal	Moraceae	1
Albizzia lebbeck	Shirish chhal	Mimosaceae	1

It is recommended in Ayurveda, combinations primarily for inflammatory conditions of the female genital tract, diabetes and anthelmintic, antimicrobial activities. In addition to these, individual drugs show anti-inflammatory, pain-reliever, hemostatic, striping action, etc. properties [6].
Majority of plant-derived active principles are polar or water-soluble in nature such as tannins, flavonoids, glycosidal aglycones possessing larger molecular size which cannot be absorbed by passive diffusion or because of their minimized lipid-solubility. Therefore, reducing their ability to pass through biological membranes which have high lipoidal content [7]. Hence, this limitation of bioavailability

can be overcome by complexing the water-soluble plant actives with dietary phospholipids which enhances oral/topical bioavailability and also preserving the clinical efficacy of phytoconstituents [8]**.** All these criteria are fulfilled by the phytosome technology. Phytosome ®, a Novel herbal drug delivery system was first established by Indena which proposes several advantages like improving the solubility of herbal actives, reducing related toxic effects, enhancement of pharmacological activities [9]. Solvent-injection method was selected over other methods for the preparation of phytosome because it offers various advantages like no need of sophisticated instruments, rapid production and easy handling, utilization of non-toxic solvents [10]. The main objective of the present study was to formulate and evaluate phytosomes of the polyherbal combination of *Cassia tora* and Panchvalkal mixture and determine its in-vitro cytotoxic activity using MTT assay against Human cervical cancer cell line (HeLa).

Materials and Methods

Soy lecithin (P-100), cholesterol were procured as gift samples from Lipoid Germany. Panchvalkal powder extracts were procured from Dr.Palep's Research Foundation Pvt. Ltd. All other chemicals used in this experiment are of laboratory grade.

1. Collection, authentication and processing of *Cassia tora* leaves

Leaves of *Cassia tora* were collected from the local market of Vasai (Maharashtra) during the month of July 2019. The authentication of fresh *Cassia tora* leaves was carried out. The sample plant twigs were identified with respect to morphology and microscopy and confirmed was identified as leaves of *Cassia tora* (Chakramarda); Subfamily: Caesalpinaceae; Family: Fabaceae.

The leaves were washed to remove soil particles or stuck particles of insecticides. The leaves were dried in a hot air oven at a temperature of 80°C to remove excess moisture. The leaves were then crushed to a fine powder and kept in air tight container.

2. Physicochemical evaluation

a. Determination of ash content

Ash content was determined as the residue remaining after incineration which are inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium, and magnesium. It was used as a criterion to judge the quality or purity of crude drugs. Total Ash value, acid- insoluble ash value and water-soluble ash value were determined using the standard procedure [11].

b. Determination of extractive value: Extractive values of crude drugs were evaluated to find out the constituents of a drug that cannot be readily estimated by any other means. Further, these values indicated the nature of the constituents present in a crude drug [12].

Procedure: About 5g of the air-dried drug, coarsely powdered was macerated, with 100ml of solvents (ethanol 95% and water) for 24 hours. The flask was frequently shaken during the first 6 hours and was allowed to stand for 18 hours. It was filtered rapidly through filter paper and the filtrate was collected. 25 ml of alcohol extract was evaporated to dryness in a tared flat-bottomed shallow dish on a water bath. The residue was dried at 105°C and weighed. It was kept in a desiccator and dried to constant weight. The % w/w of extractive value for different solvents with reference to the air-dried drug was calculated [3].

3. Preparation of plant extracts [13]

Extraction was carried out using the Soxhlet apparatus. 20 gm. of dried powder and was filled in a thimble holder which was placed in an extracting chamber. Initially, it was subjected to defatting with petroleum ether 60:80 as defatting solvent with continuous cycles for 6-7 hours per day for 3-4 days. This was carried out until a clear, colourless solvent is obtained in the siphon tube. Defatting was done to remove fats, waxes, pigments such as chlorophyll. After completion of defatting, the remaining marc was extracted with ethanol. Extracting solvent was condensed for the solvent recovery. A portion of solvent was evaporated on the electrical water bath. Drying is done to minimize bacterial growth, increase chemical stability and obtain the required concentrate.

4. Phytochemical evaluation

The extract was subjected to phytochemical evaluation to determine the presence of various phytoconstituents *viz.* flavonoids, terpenoids, alkaloids, glycosides, saponins, tannins, phenols, carbohydrates and steroids.

5. Preformulation studies

UV-Vis spectrophotometry analysis of the combination of *Cassia tora* and panchvalkal

The stock solution of 200µg/ml was prepared by dissolving 10mg of *Cassia tora* ethanolic extract and 10mg of Panchvalkal mixture in 100ml of 1:1hydroalcoholic solvent. The prepared solution was scanned in 200- 600nm range using UV-Vis spectrophotometer. Standard calibration curve was arranged with serial dilutions employing the stock solution.

6. Preparation of phytosomes of the combination of ethanolic extract of *cassia tora* and panchvalkal mixture:

Solvent injection technique was used (Figure 1) (Table 2).

1. The drug extract was dissolved in 5ml of 1:1 hydroalcoholic solvent. Cholesterol and lipid (Soy lecithin) were dissolved in ethanol. This solution was sonicated till it formed a clear solution.
2. The drug extract solution was treated as an aqueous phase and was made upto volume with the remaining Phosphate buffer pH 7.0. The lipid phase was added dropwise to the aqueous phase and kept for stirring using the magnetic stirrer at 1300rpm for 2 hrs.
3. Tween-80 was added as a surfactant to reduce the interfacial tension between the aqueous and lipid phase and stirring was continued.
4. Several batches were prepared using different ratios of excipients based on the trial and error method depending on the achievement of maximum entrapment efficiency.

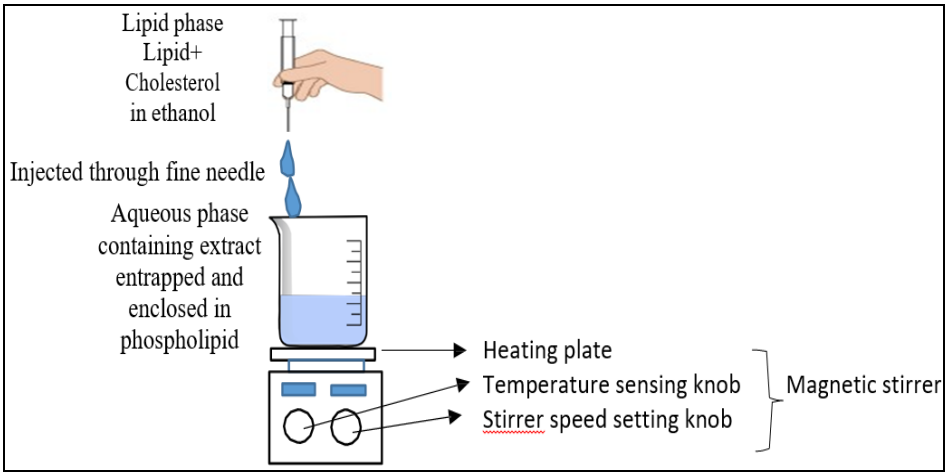

Fig 1: Solvent injection method for preparation of phytosome

Table 2: Preparation of phytosome of combination of panchvalkal and *C.tora*

Formulation No.	Panchvalkal mixture (mg)	c. *tora* (mg)	Total extract (mg)	Lipid-100 (mg)	Cholesterol (mg)	PB pH 7.0 (ml)	Ethanol (ml)	Extract: Lipid: Cholesterol ratio
F1	5	5	10	10	4	15	5	1:1:0.4
F2	5	5	10	10	6	15	5	1:1:0.6
F3	5	5	10	20	8	15	5	1:2:0.8
F4	5	5	10	40	5	15	5	1:4:0.5
F5	5	5	10	45	5	15	5	1:4.5:0.5
F6	5	5	10	45	8	15	5	1:4.5:0.8
F7	5	5	10	50	5	15	5	1:5:0.5
F8	5	5	10	55	5	15	5	1:5.5:0.5
F9	40	40	80	60	10	15	5	8:6:1
F10	50	50	100	80	20	20	5	10:8:2

Evaluation of phytosome [7]

1. **% Entrapment efficiency**

Entrapment efficiency of phytosomal batches was determined using the centrifugation method. The prepared phytosomes were placed in an eppendorf tube and centrifuged at 12000 rpm for 30 minutes at 4°C in a cooling centrifuge (REMI ultra-centrifugation) for settling down of vesicles. The drug present freely in the supernatant was used to find out the amount of unentrapped drug. 1: 10 dilution of supernatant was made with phosphate buffer (pH 7.0). Absorbance was taken at lambda max using buffer as blank. The absorbance value was put in the calibration curve to determine the concentration of the free drug. % Entrapment efficiency was determined using the following equation

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug-Amount of unentrapped drug}}{\text{Total drug}} X100$$

2. **Particle size and zeta potential measurement-** Determination of particle size and zeta potential were carried out which is a critical parameter for the stabilization and should comply within the set ranges. Particle size measurement and polydispersity index of the prepared phytosome formulation were carried out using Malvern Zetasizer Version 7.04 Serial No. MAL1099365 Zeta potential analysis was carried out using HORIBA Scientific SZ-100 Version 2.0.

3. ***In-vitro* release studies**

Drug release profiles of nano-sized drug delivery systems like nanoparticles, liposomes, nanosuspensions and emulsions can be determined by membrane diffusion (i.e. dialysis) method which is considered as one of the most convenient techniques. The dialysis offers advantages over other methods such as ease of sampling and enables efficient replacement of receptor media due to the barrier separation of the formulation (by semipermeable dialysis membrane) from the receptor media [14].

Procedure- The in-vitro diffusion release study was carried out in a 250ml beaker containing 150 ml of diffusion medium phosphate buffer solution pH 7.0. A previously overnight soaked dialysis membrane in the diffusion medium was used for the study. As a barrier to isolate between donor and receptor phase, this dialysis membranewas used. One end of dialysis membrane measuring 10cm was taken and was sealed with the help of thread. From another end, phytosome formulation 2ml was poured into the dialysis membrane and tied with thread. Phytosome enclosed dialysis membrane was suspended into the medium. Beaker was mounted on a magnetic stirrer and equilibrated at 37°C. Content of beaker was kept to stirred at 100rpm and aliquots were withdrawn at different time intervals for a period of 24 hours and replaced by 5 ml volume of phosphate buffer pH 7.0 to maintain the sink condition. Absorbance of these samples was analyzed by UV Spectrophotometer at λmax 264nm.

4. **Physical stability studies** [15]

Stability studies were performed by storing the formulations at 4°C and 25°C (Room temperature) for 6months. The formulations were evaluated on basis of appearance, clarity, sedimentation, phase separation, gelation, and/or color change. Moreover, drug entrapment evaluation was done for stored samples as mentioned earlier.

***In-vitro* cytotoxicity evaluation of phytosomal of combined ethanolic extract of *cassia tora* and panchvalkal mixture on human cervical cancer cell line (HeLa).**

The samples for anticancer activity were tested in averin biotech Pvt. Ltd.

Maintenance of Cell Lines

The HeLa (Human Cervix adenocarcinoma cell line) is purchased from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic- antimycotic solution in the atmosphere of 5% CO2, 18-20% O2 at 370C temperature in the Co2 incubator and subcultured for every 2days.

Background of the Study

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on the reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. The mitochondrial lactate dehydrogenase produced by living cells reduces MTT to insoluble formazan crystals. When dissolved in a suitable solvent, it appears purple and its intensity is proportional to the number of viable cells and can be measured spectro photo metrically at 570nm. [16, 17]

Materials

1. Cell lines: HELA - human cervix adenocarcinoma cell line (From NCCS, Pune)
2. Cell culture medium: DMEM (Dulbeco's Modified Essential Media)-High glucose media - (Cat No:2120785, Gibco)
3. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
4. Fetal Bovine Serum (#RM10432, Himedia)
5. MTT Reagent (5 mg/ml) (# 4060 Himedia)
6. DMSO (#PHR1309, Sigma)
7. Cisplatin (#PHR1624, Sigma)
8. D-PBS (#TL1006, Himedia)
9. 96-well plate for culturing the cells (From Corning, USA)
10. T25 flask (# 12556009, Biolite - Thermo)
11. 50 ml centrifuge tubes (# 546043 TORSON)
12. 1.5 ml centrifuge tubes (TORSON)
13. 10 ml serological pipettes (TORSON)
14. 10 to 1000 ul tips (TORSON)

Equipments

1. Centrifuge (Remi: R-8oC).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted microscope (Biolink)
4. 37°C incubator with humidified atmosphere of 5% CO2 (Healforce, China)

Assay Controls

1. Medium control (medium without cells)
2. Negative control (medium with cells but without the experimental drug/compound)
3. Sitive control (medium with cells and 15uM of Cisplatin)

Note: The culture media containing extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol might reduce the MTT to formazan. It is important to use the same medium in control as well as test wells to account for this reduction.

Steps Followed

1. 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well) was seeded without the test agent. The cells were allowed to grow for about 24 hours.
2. Appropriate concentrations of the test agent was added i.e. i.e. 6.25, 12.5,25,50,100 µg/ml.
3. The plate was incubated for 24 hrs. at 37°C in a 5% CO2 atmosphere.
4. After the incubation period, the plates were taken out from the incubator and spent media was removed and MTT reagent was added to a final concentration of 0.5mg/mL of total volume.
5. The plate was wrapped with aluminium foil to avoid exposure to light.
6. The plates were returned to the incubator and incubated for 3 hours. (Note: Different cell lines incubate at a different rate. Within one experiment, incubation time should be kept uniform while making comparisons.)
7. MTT reagent was removed and then 100 µl of solubilization solution (DMSO) was added.
8. Gentle stirring in a gyratory shaker will enhance dissolution. For complete solubility of the MTT formazan crystals, pipetting up and down maybe occasionally required especially in dense cultures.
9. Absorbance was read on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.
10. The IC50 value was determined by using linear regression equation i.e. Y =Mx+C.

Here, Y = 50, M and C values are obtained from the viability graph.

Formula Used for the Study

$$\% \text{ Cell Viability} = \frac{\text{Mean Absorbance of Sample@ 570nm}}{\text{Mean absorbance of Untreated@570nm}} * 100$$

Results and Discussion

Physico-chemical evaluation

Ash value [11]: After incineration of vegetable drugs, they leave inorganic ash which can be naturally found in the drug or adhering to it or deliberately added to it, in the form of adulteration. Low-grade products, exhausted products, drugs with an excess of sandy or earthy matter can also be detected by this method. The total ash contains majorly carbonates phosphates, silicates, and silica. The total ash of the crude powder of *Cassia tora* leaves was found to be 21% w/w (Table 3). Acid-insoluble ash is the residue obtained after extracting the total ash with HCl. It gives an idea about the amount of silica present in earthy matter. Acid-insoluble ash of crude powder of *Cassia tora* leaves appeared to be 2.5%w/w (Table 3). Water-soluble ash is the amount of ash which is soluble in water. 8.87%w/w of water-soluble ash occurred in*Cassia tora* leaves (Table 3).

Extractive value: Degree of quality, purity, and adulteration due to exhausted and incorrectly processed drugs can be determined by extractive values with different

solvents. 21 %w/w was obtained as ethanol-soluble extractive value (Table 3). Water-soluble extractive value is applied for the drugs which contain water-soluble constituents like tannins, sugars, plant acids and mucilage. 15 %w/w of water-soluble extractive value was acquired from *Cassia tora* leaves (Table 3).

Table 3: Physico-chemical properties of *Cassia tora* leaves

Sr. No.	Physico-chemical properties	Results (%w/w)
1.	Ethanol soluble extractive value	21
2.	Water soluble extractive value	15
3.	Total ash	15.55
4.	Acid insoluble ash	2.5
5.	Water-soluble ash value	8.87

Phytochemical evaluation

Preliminary phytochemical analysis of ethanolic extract of *Cassia tora* leaves revealed the presence of alkaloids, terpenoids, Saponins, carbohydrates, anthraquinone glycosides.

Preformulation studies

UV-Vis spectrophotometry analysis of combination of *Cassia tora* and panchvalkal

Fig. 2 represents UV-Vis scan of 200µg/ml of the combination of ethanolic extract of leaves of *Cassia tora* and Panchvalkal mixture in 1:1 hydroalcoholic solvent. The maximum wavelength was found to be 264nm.

Preparation of Standard Calibration curve: The stock solution having final concentration of 200µg/ml was used to get solutions of varying concentrations ranging from, 20µg/ml, 40 µg/ml, 60µg/ml, 80µg/ml, 100µg/ml, 120 µg/ml, 140 µg/ml, 160 µg/ml 180 µg/ml, 200 µg/ml. The absorbance of these solutions was observed at 264nm (Figure 2).

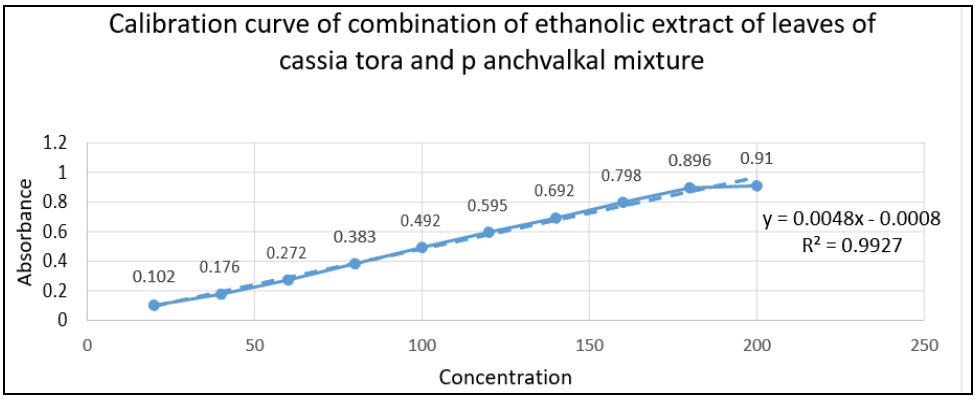

Fig 2: Calibration curve of combination of ethanolic extract of leaves of *Cassia tora* and panchvalkal mixture

Evaluation of Phytosome Complex

% Entrapment efficiency

F9 showed maximum drug entrapment efficiency of 73.45%. The graph reveals that with the increase in lipid and cholesterol ratio, the entrapment efficiency % increased concluding that lipid plays a significant role in entrapping the drug. The addition of cholesterol reduces the leakage rate from the phosphatidylcholine layer [18]. After a certain ratio, the % E.E reduced which concludes that further increasing the lipid ratio doesn't facilitate in increasing the % E.E (Figure 3).

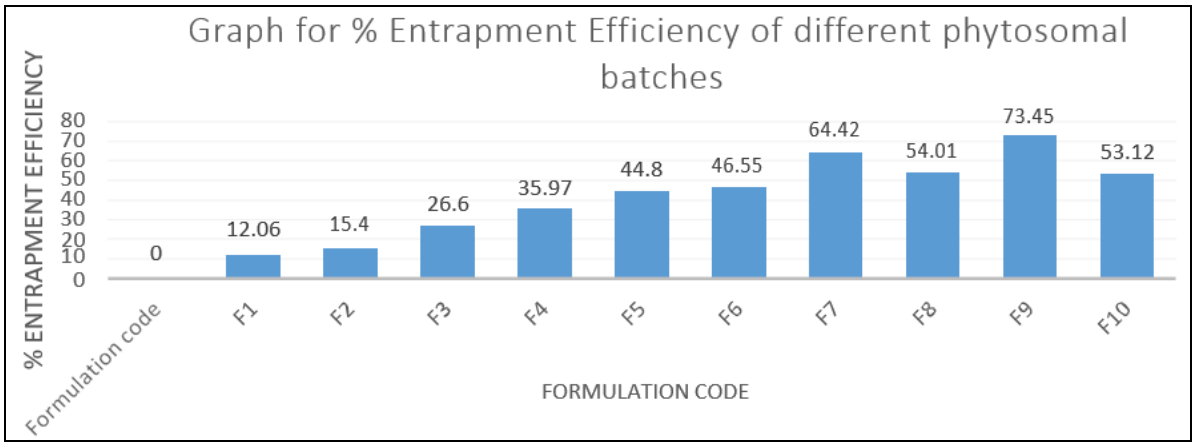

Fig 3: Graph for % Entrapment Efficiency of different phytosomal batches

Measurement of particle size

Particle size of the drug affects the rate of absorption [19]. Particle size possess a significant function in developing process stages and quality control of particle systems to attain satisfactory efficient methods and achieve high final product quality. The particle size of the optimized batch was found to be 473.9 nm (Figure 4).

"Polydispersity" also referred as the heterogeneity index, is used to identify the degree of non-uniformity of a size distribution of particles. The PDI index ranges from 0.0 (for

a sample consisting of totally uniform particle size) to 1.0 (for a highly poly disperse sample with multiple particle size populations) [20]. PDI of the phytosome complex was found to be 0.417 (Figure 4).

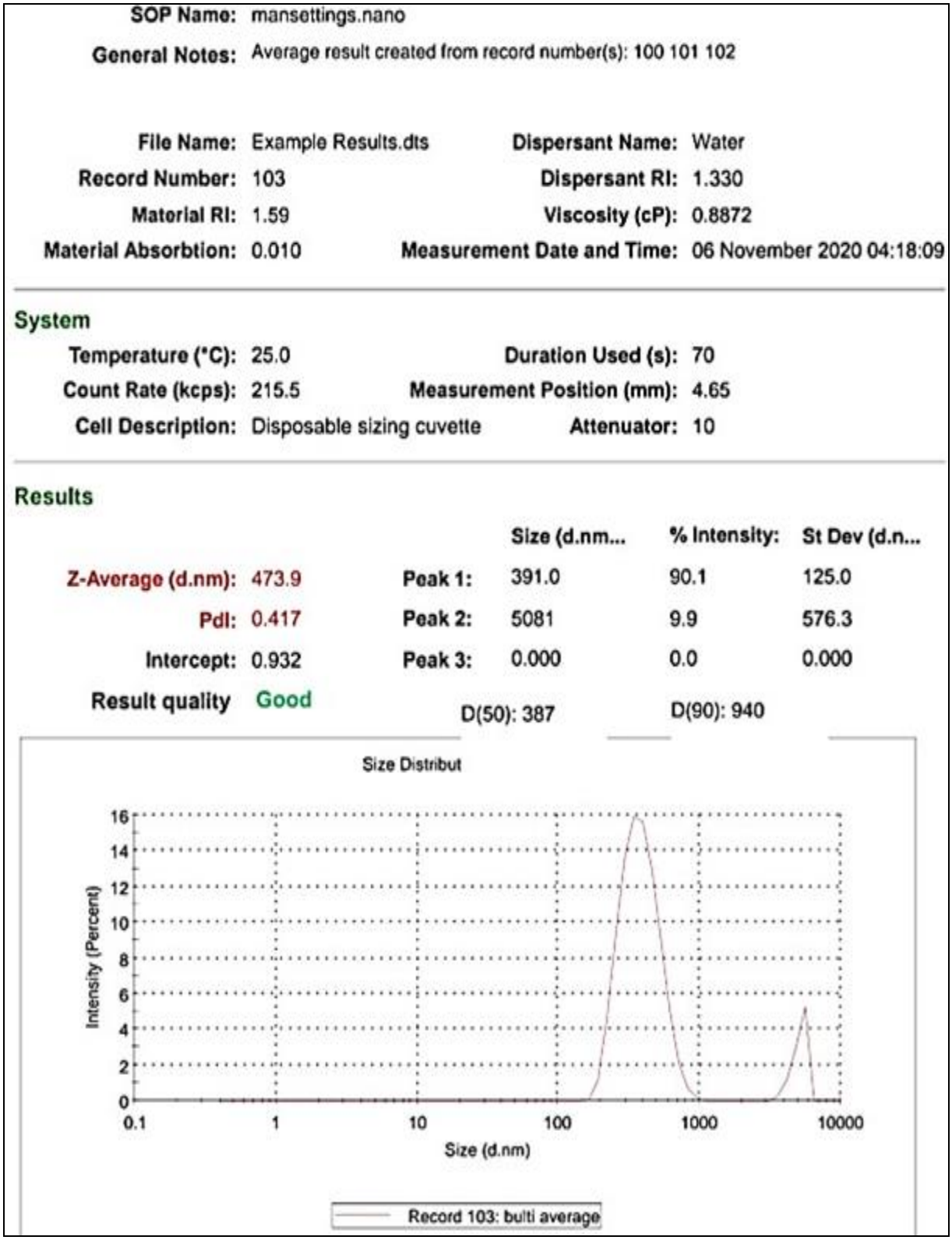

Fig 4: Particle size analysis of phytosome

Measurement of zeta potential

Zeta potential is one of the vital properties of the particle that can influence the particle stability.

It refers to the electrostatic repulsion between particles with the same electric charge which results in separation of the particles and thus avoids adhesion among particles and consequently preventing flocculation and sedimentation [21]. The Zeta potential of the phytosome complex was found to be -16.4mV (Figure 5).

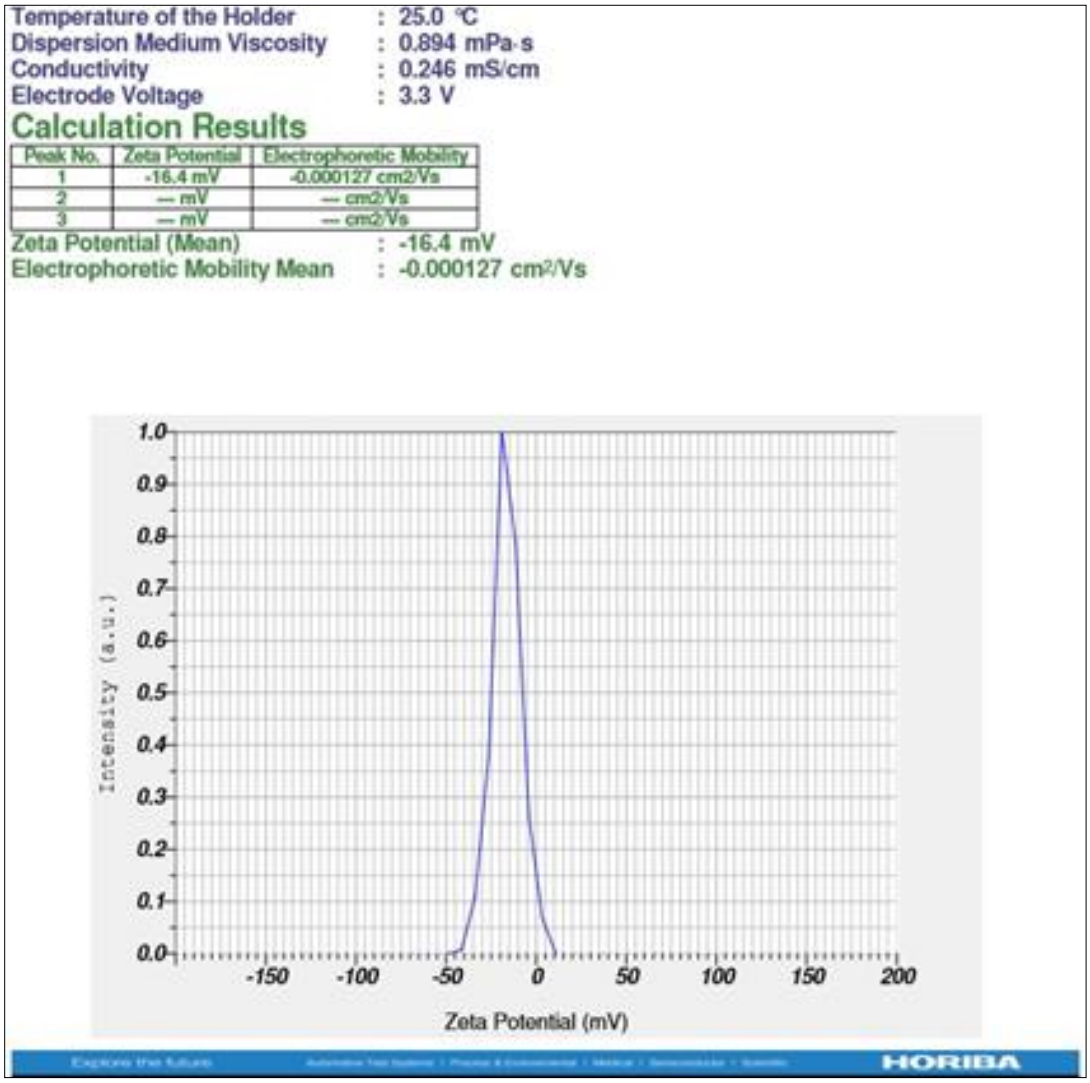

Fig 5: Zeta potential of phytosome

***In-vitro* release studies**

Dialysis method was used for the in-vitro release studies. Comparative release studies were done for extract, marketed formulation, and phytosome for a period of 24hrs. The optimized batch F9 was proved to be the best formulation with 73.45% of drug entrapment and 74.86% of drug release (Figure 6).

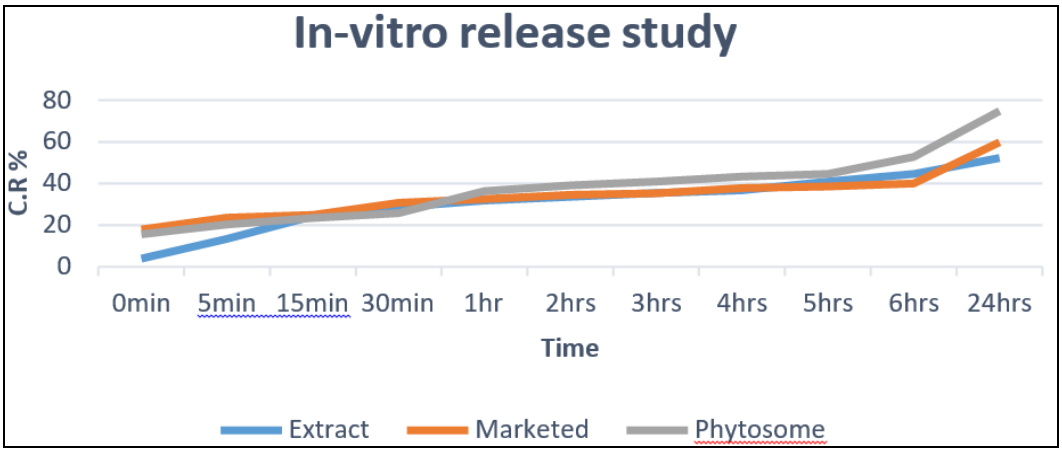

Fig 6: In-vitro release comparison study for phytosome complex F9

Physical stability studies

Physical stability studies of phytosome was conducted by storing them at different temperature ranges 4°C ± 2°C, 25°C± 2°C for a period of 6 months and studied for visual inspections as well as the physical stability parameters and drug entrapment. The batches still appeared clear and no sedimentation was observed (Table 4).

Table 4: Physical stability studies of optimized F9 phytosome

Sr. No.	Physical stability parameters		Storage temperature	
			4°C	25°C
1.	Clarity test		Pass	Pass
2.	Sedimentation		None	None
3.	Phase separation		None	None
4.	Gelation		None	None
5.	Color change		None	None
6.	Drug entrapment	Initial	73.45%	73.45%
		3 months	71.02%	69.83%
		6 months	66.14%	64.79%

***In-vitro* cytotoxicity evaluation of combined ethanolic extract of *cassia tora* and panchvalkal mixture and their phytosomal formulation on human cervical cancer cell line (HeLa).**

After performing experiment, the observations obtained are given in table as follows:

Extract

Table 5: % Cell viability for different concentrations of extract

Concentration Unit: µg/ml					Incubation: 24hrs			
Blank		Untreated	Std	6.25	12.5	25	50	100
Reading 1	0.041	0.761	0.381	0.725	0.684	0.613	0.537	0.375
Reading 2	0.047	0.768	0.389	0.72	0.691	0.608	0.54	0.382
Mean	0.044	0.764	0.385	0.722	0.6875	0.6105	0.5385	0.3785
Mean OD (Sample-Blank)		0.7205	0.341	0.6785	0.6435	0.5665	0.4945	0.3345
Standard Deviation		0.0049497	0.00565	0.003535	0.004949747	0.003535534	0.00212132	0.00495
Standard Error		0.0035	0.004	0.0025	0.0035	0.0025	0.0015	0.0035
Cell Viability %		100	47.3282	94.17071	89.3129771	78.6259542	68.63289382	46.42609

IC 50 VALUE= 90.40ug/ml

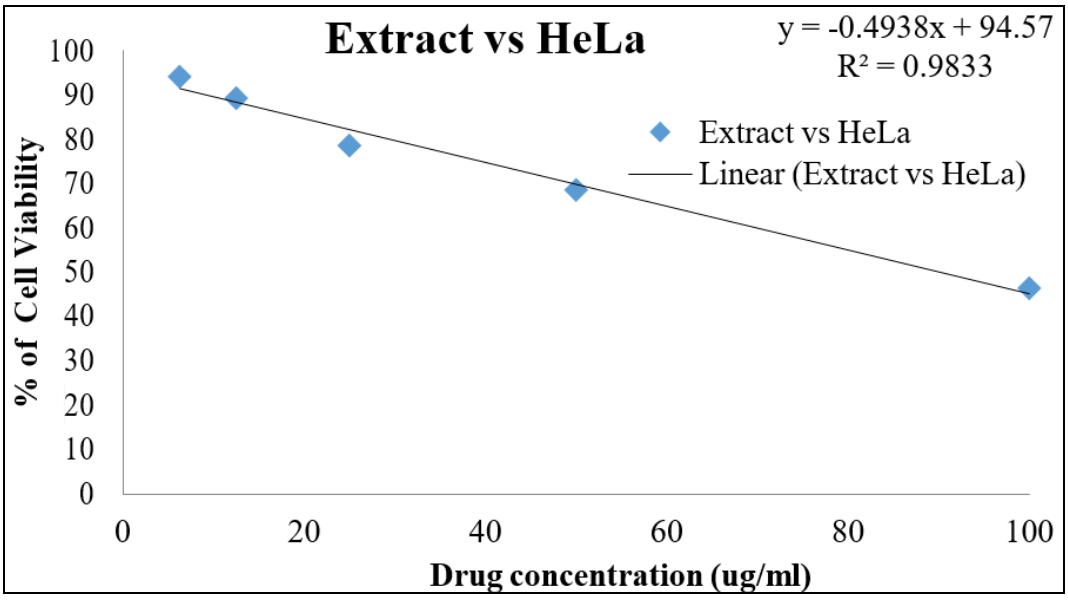

Fig 7: Graph for Drug concentration vs. % of cell viability for extract

F9

Table 6: % Cell viability for different concentrations of F9

Concentration Unit: µg/ml	Incubation: 24hrs							
	Blank	Untreated	STD	6.25	12.5	25	50	100
Reading 1	0.041	0.761	0.381	0.702	0.617	0.458	0.326	0.159
Reading 2	0.047	0.768	0.389	0.693	0.615	0.454	0.319	0.167
Mean	0.044	0.7645	0.385	0.6975	0.616	0.456	0.3225	0.163
Mean OD (Sample- Blank)		0.7205	0.341	0.6535	0.572	0.412	0.2785	0.119
Standard Deviation		0.00494974	0.00565685	0.006363961	0.0014	0.0028	0.00494	0.00565
Standard Error		0.0035	0.004	0.0045	0.001	0.002	0.0035	0.004
Cell Viability %		100	47.32824427	90.70090215	79.389	57.182	38.6537	16.5163

IC 50 VALUE= 32.03ug/ml

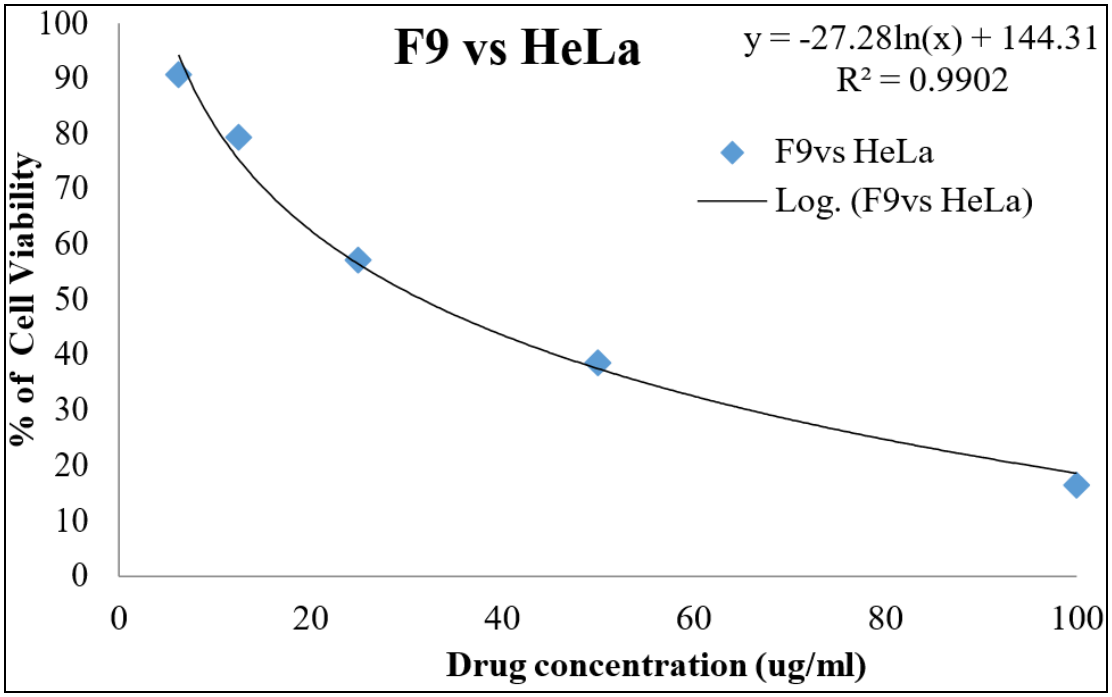

Fig 8: Graph for Drug concentration vs. % of cell viability for F9

F10

Table 7: % Cell viability for different concentrations of F10

Concentration Unit: µg/ml	Incubation: 24hrs							
	Blank	Untreated	STD	6.25	12.5	25	50	100
Reading 1	0.041	0.761	0.381	0.737	0.692	0.626	0.548	0.315
Reading 2	0.047	0.768	0.389	0.746	0.685	0.619	0.553	0.311
Mean	0.044	0.7645	0.385	0.7415	0.6885	0.6225	0.5505	0.313
Mean OD (Sample- Blank)		0.7205	0.341	0.6975	0.6445	0.5785	0.5065	0.269
Standard Deviation		0.00494974	0.0056568	0.0063639	0.004949	0.004949	0.003535	0.002828
Standard Error		0.0035	0.004	0.0045	0.0035	0.0035	0.0025	0.002
Cell Viability %		100	47.328244	96.807772	89.45176	80.29146	70.29840	37.33518

IC 50 VALUE= 79.83ug/ml

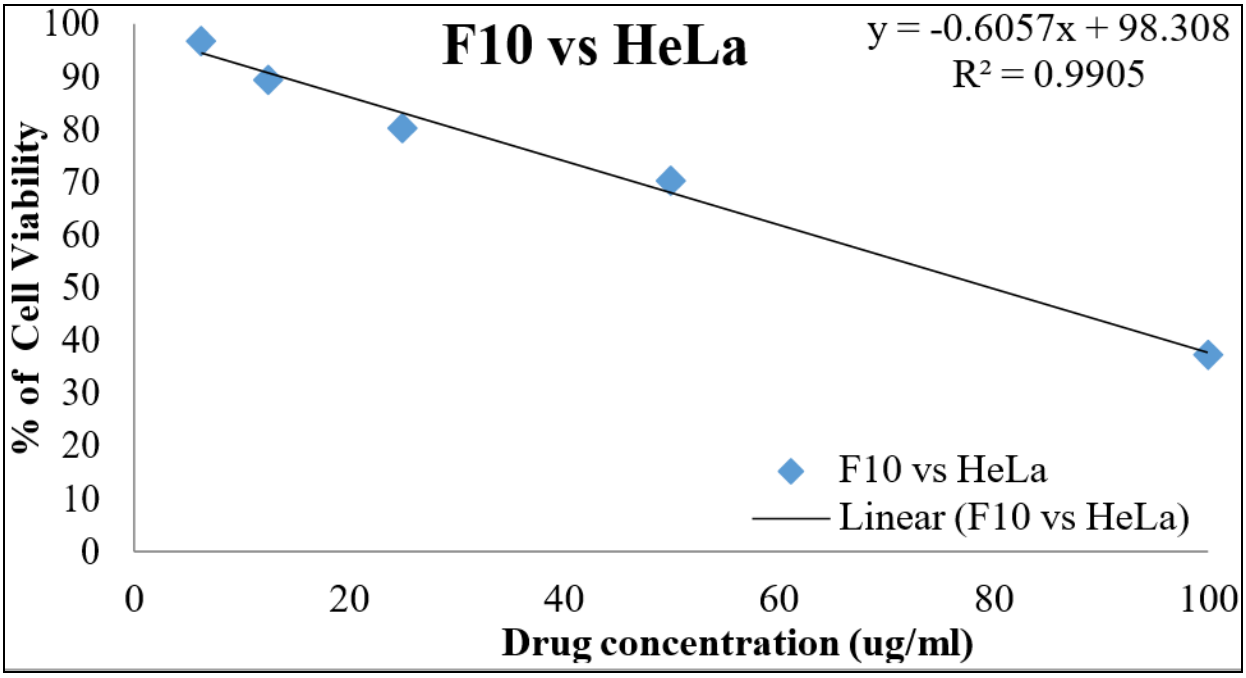

Fig 9: Graph for Drug concentration vs. % of cell viability for F10

Table 8: showing the IC50 concentrations of the Test Compounds extract, F9 and F10 treated with HeLa cell lines for incubation period of 24hrs.

S. No	Sample	Test Parameter-MTT
		IC50 (ug/ml)
1	Extract	90.40
2	F9	32.03
3	F10	79.83

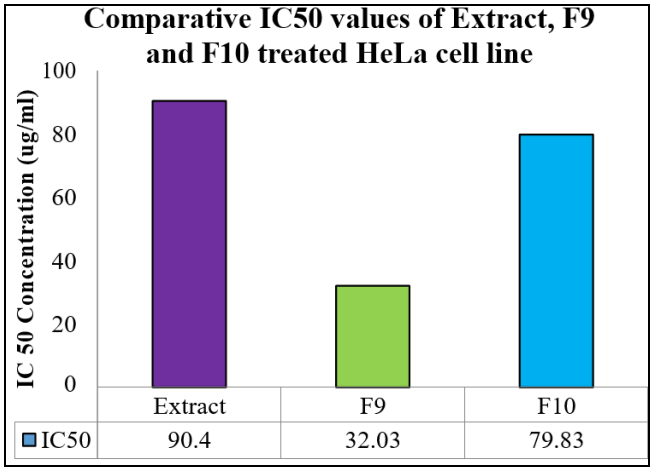

Fig 10: Overlaid Bar graph showing the IC50 values of extract, F9 and F10 on HeLa cell lines by MTT study.

Discussion

The Observations in Statistical data of cell cytotoxicity Study by MTT suggesting us that against HELA cell lines, Test Compounds namely Extract, F9 and F10 showing significant cytotoxic potential properties with the IC50 Concentrations at 90.4ug/ml, 32.03ug/ml and 79.83ug/ml respectively compared to the standard drug used for the study. Among all these compounds, F9 may be considered as a potent anti- cancer agent due to its low IC50 value on HELA cells.

Further studies like Cell Cycle Study by PI staining, Apoptosis study by Annexin V/PI staining, Apoptotic Protein expressions like Caspase 3,7,9, Bcl2,p53 and ROS study to evaluate the mechanism of action of test compounds *viz*., Form Powder and F5 behind the anticancer potential in in-vitro conditions.

Conclusion

Numerous investigations and research studies reported anticancer activity of *Cassia tora* and Panchvalkal against Human cervical cancer cell line (HeLa). The objective of the study was to combine them and formulate them into phytosome, a novel drug delivery system which enhances the absorption and bioavailability of water-soluble plant actives. Solvent injection method was employed for the preparation of phytosome using phosphatidylcholine and cholesterol. The formulation thus made was optimized for maximum entrapment efficiency. The formulated phytosome was screened for anticancer activity which concluded that the phytosome showed significant anticancer activity. Thus, the phytosome of the combination of *Cassia tora* and Panchvalkal is useful in the prevention of malignant transformation.

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