Optimized *Terminalia arjuna* Phytosomes: A Promising Approach for Cardiovascular Health

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Abstract:

Cardiovascular diseases continue to pose a significant threat to global health, necessitating innovative therapeutic strategies. In this study, we focused on harnessing the potential of Terminalia arjuna, a revered medicinal plant in Ayurvedic medicine, for cardiovascular applications. We formulated and characterized Terminalia arjuna phytosomes, utilizing a unique approach to enhance their bioavailability and efficacy.

The study involved a comprehensive extraction process, and the development of phytosomes using a solvent injection method. Quality by Design (QbD) principles guided the optimization process, ensuring the formulation's stability and effectiveness. Various analytical techniques, including UV-Vis spectroscopy, DSC, and FTIR, were employed to characterize the phytosomal formulation.

Our results demonstrated that the optimized phytosomes exhibited superior stability, a favorable particle size, and high entrapment efficiency. Furthermore, the formulation displayed sustained release properties, potent antioxidant activity, and significant pro-angiogenic effects, highlighting its potential therapeutic benefits for cardiovascular health. The pro-angiogenic activity observed in the chick chorioallantoic membrane (CAM) assay suggests the formulation's ability to promote blood vessel formation, crucial for tissue repair and healing.

In conclusion, our study presents a novel approach to harnessing the medicinal properties of Terminalia arjuna through phytosomal formulation. The optimized phytosomes show promising potential as a therapeutic agent for cardiovascular diseases, addressing both antioxidant and pro-angiogenic aspects. This research opens new avenues for herbal medicine, emphasizing the importance of enhancing bioavailability and stability for maximizing the therapeutic impact of natural compounds.

Keywords: *Terminalia arjuna*, Phytosome, Analytical evaluations, Kinetic studies, Pro-angiogenic activity

1. Introduction:

Terminalia arjuna is a perennial medicinal tree belonging to the family Combretaceae found in an Asian region fig no 1. It is highly valued in Ayurvedic medicine for its potent cardioprotective activity. Along with its cardioprotective activity, it is useful in ecchymosis, spermatorrhoea, and sexually transmitted diseases such as gonorrhea. Terminalia arjuna has an astringent, cooling, and aphrodisiac, and is used for the treatment of cough, leucorrhoea, excessive perspiration, ulcers, diabetes, tumors, asthma, inflammation, and many skin disorders(1). It is one of the most resourceful medicinal plants with a wide range of biological actions. The biological activity of Arjuna is due to the presence of various biologically active constituents in its bark. The bark of the Arjuna tree contains several active compounds, including triterpenoids, flavonoids, tannins, and minerals such as calcium and magnesium. Most of these constituents of plants are polar and water-soluble. However, water-soluble phytoconstituents like flavonoids, tannins, glycosides aglycones, etc. are poorly absorbed due to their large molecular size, which cannot be absorbed by passive diffusion, or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability(2). Phytosomes are a type of herbal extract that are bound to phospholipids, which are the same type of molecules that make up cell membranes in the human body(3). This unique structure enhances the absorption and bioavailability of the active compounds in the herbal extract. The importance of phytosomes lies in their ability to improve the effectiveness of herbal supplements(4). In traditional herbal extracts, the active compounds are often poorly absorbed by the body and can be quickly eliminated, limiting their therapeutic potential. By binding the herbal extract to phospholipids, the phytosomes, Thus formed can increase the absorption and bioavailability of the active compounds, allowing for more effective delivery and better health outcomes(5).

In addition to improving absorption, phytosomes may also enhance the stability and shelf life of herbal extracts. The phospholipid coating can protect the active compounds from degradation and oxidation, ensuring that the supplement remains potent and effective(6).

Hence, the current study aimed at improving the release characteristics of *Terminalia arjuna* extract by formulating a *Terminalia arjuna* phytosomes tablet. *Terminalia arjuna Linn*. belongs to the family Combretacae(7). *Terminalia arjuna* bark contains various phytoconstituents like saponins, L-tocopherol, ascorbic acid, flavonoids, alkaloids, cardiac glycoside, tannins. Flavanoids include quercetin, baicalein, luteolin,arjunolone, Kempferol etc. Arjuna bark is often used for its potential benefits in supporting cardiovascular health. It is speculated to have anti-inflammatory, antioxidant, and lipid-lowering effects(8). It might help lower blood pressure, strengthen cardiac muscles, and enhance heart function. Along with that, it may promote the formation of blood vessels(9). In this study, a complex of *Terminalia arjuna* alcoholic extract and lipids were prepared, and the physiochemical properties and Pro-angiogenic activity of extract and phytosomal complex were evaluated by CAM assay.

Fig 1 *Terminalia arjuna* tree

2. Materials and method:

Materials:

Soya lecithin was procured as a gift sample from Lipoid Germany. *Terminalia arjuna* Linn. drug was procured from Dr. Palep's Research Foundation Pvt. Ltd. Ambernath. All other chemicals and reagents used were of laboratory grade.

Method:

Extraction and yield optimization: Authentication of *Terminalia arjuna* was carried out in specimen no p 019215853 matched with the pharmacognostic feature of standard *Terminalia arjuna* family Combretaceae. Optimizing yield in extraction processes involves maximizing the number of target compounds or substances obtained from raw material. Maceration, Soxhlet, and ultrasonication methods of extraction were employed to improve yield, depending on the nature of the material and the desired compounds.

Selection of λ max:

A 100-ppm (10mg/100ml) concentration solution was prepared by dissolving ethanolic extract in DMSO and phosphate buffer of PH 5.5 The solution was scanned in UV-Vis spectra between 200 and 800 nm using phosphate buffer (PH 5.5) as a blank(10).

TLC analysis: The pure ethanolic extract of *Terminalia arjuna* failed to yield a favourable result, the ethanolic extract must be hydrolysed in order to quantify the amount of quercetin in the extract. Conc. HCL was used for the hydrolysis of the sample. The extract and Conc. HCL were refluxed for 4 hrs. Obtained filtrate was treated with ethyl acetate to separate out the flavonoids and glycoside content of the extract

Preparation of standard calibration curve of ethanolic extract of *Terminalia arjuna*:

A stock solution of 1000 µg/mL was prepared by dissolving 10 mg of *Terminalia arjuna* ethanolic extract in phosphate buffer at pH 5.5. The standard solutions with concentrations ranging from 20-100 µg/mL were obtained from the stock solution. The absorbance of these solutions was measured at a wavelength of 254 nm, in duplicate, using phosphate buffer at pH 5.5 as a blank. The absorbance values were utilized in the preparation of the standard plot(11).

Preparation of Phytosomes containing *Terminalia arjuna* ethanolic extract:

The solvent injection approach was employed to manufacture phytosomes of *Terminalia arjuna* ethanolic extract. A solution was prepared by dissolving several concentrations of lipids, specifically soya lecithin, and cholesterol, in 5 ml of ethanol. In a separate container, the ethanolic extract derived from *Terminalia arjuna* was solubilized in 3 ml of dimethyl sulfoxide (DMSO) and subsequently diluted to a total volume of 10 ml using a phosphate buffer with a pH of 5.5. This resulting solution is referred to as the aqueous phase. The lipid phase was introduced into the aqueous phase using a syringe, with a single injection performed at a rotational speed of 500 revolutions per minute (rpm). Following the conclusion of the addition process, the mixture underwent stirring for a duration of three hours at a rotational speed ranging from 12,000 to 13,000 revolutions per minute (rpm) utilizing a magnetic stirrer. This stirring procedure was employed to facilitate the evaporation of the solvent and achieve a homogeneous vesicular dispersion. The phytosomal sample was retained for the purpose of conducting sedimentation analysis. If sedimentation does not occur, the batch has the potential to remain stable. The ultimate composition underwent freeze-drying, employing a cryoprotectant consisting of D-(+)-Trehalose Dihydrate at a concentration of 5% w/v(11).

Optimization by applying QbD approach:

Depending upon preliminary studies and screening factors, CMAs influencing the quality of the product were selected for optimizing the formula systematically. design was selected based on the results obtained from the screening of factors with the lowest and highest value. The selected factors were Concentration of Lipid (mg), Concentration of cholesterol (mg), and stirring time which ultimately show an impact on the Product quality profile. 2^3full factorial design was selected to analyze the effect of CMAs and CPP on CQAs in which it presents 8 runs. ANOVA and Contour plots were performed with particle size and Entrapment Efficiency parameters. optimization of formulation done using DOE software.

Characterization of *Terminalia arjuna* Phytosomes:

Measurement of Particle size and zeta potential: The average particle size was analyzed by the Nanoparticle tracking analysis (NTA) version 2.3 and zeta potential was analyzed by the Malvern V2.2 instrument. For the measurement, the formulation was diluted with an appropriate volume of double distilled water(12).

Determination of entrapment efficiency: The calculation of this parameter was conducted in order to determine the quantity of ethanolic extract that was encapsulated within the phytosomes. The unencapsulated ethanolic extract was quantified by subjecting the phytosomal formulation to centrifugation at a speed of 15000 revolutions per minute for a duration of 1 hour. Following the process of centrifugation, the resulting supernatant was subjected to analysis using a UV-visible spectrophotometer set at a wavelength of 254 nm. The calculation of entrapment efficiency was performed using the following formula.

Entrapment Efficiency (%) = Weight of total drug – Weight of free drug / Weight of total drug(13).

Differential scanning Calorimetry DSC: The ethanolic extract of *Terminalia arjuna*, Soya lecithin, it and the phytosomes complex were precisely measured and placed in a standard aluminum pan. The pan was then sealed tightly and subjected to heating from 0 ºC to 300 ºC at a constant rate of 10 ºC per minute. Throughout the heating process, a constant flow of nitrogen at a rate of 20 ml per minute was used to purge the system. This experiment was conducted using a Mettler Toledo DSC 60 instrument equipped with STAR®SW 9.20 data recording software. The analyzer was utilized to record the temperatures at which peak transitions occurred(14).

FTIR: Fourier Transform Infrared (FTIR) spectral data were acquired using a Shimadzu instrument from Japan. The purpose of this analysis was to determine the structure and chemical stability of the *Terminalia arjuna* extract, soya lecithin, cholesterol, and phytosomes complex. The samples were analyzed using the Attenuated Total Reflectance Infrared (ATR IR) method under varying pressure conditions. Spectral scanning was conducted within the wavelength range of 4000 to 400 cm-1(15).

In-vitro Release studies of extract and phytosomal formulation: The In-vitro release study was conducted using a Franz diffusion cell and a Dialysis membrane-50 with a molecular cut-off of 12000 to 14000 Da. The release experiments were performed in a phosphate buffer solution with a pH of 5.5. The phytosomes that had been manufactured, with a volume of 3 ml, were placed into the donor compartment. The contents were agitated at a rotational speed of 100 (rpm) under ambient temperature conditions. Samples of 3 mL were extracted at various time intervals. The fresh medium was replenished by withdrawing an equal volume of the sample. The aliquots were subjected to analysis at a wavelength of 254 nm using a UV-visible spectrophotometer. The percentage of drug release was then determined at various time intervals(16).

Stability Studies: The phytosomes formulations that were optimized were subjected to storage at various temperature ranges, namely 4ºC ± 2ºC, 25ºC ± 2ºC. The physical stability of the manufactured phytosomes was assessed over a period of 6 months, with a focus on evaluating the drug entrapment efficiency.

In vitro antioxidant activity evaluation of extract and phytosomes: Antioxidant activity: The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the antioxidant activity of the extract. The principle behind the DPPH assay is based on the ability of antioxidants to scavenge the stable free radical DPPH.

In order to conduct the DPPH antioxidant test, it is necessary to prepare a 0.1mM DPPH solution with methanol as the solvent. A calibration curve was established using ascorbic acid, an ethanolic extract of Terminalia arjuna, and a phytosomal batch of varying concentrations ranging from 20 to 100 ppm. In each individual test tube, mix 4 ml of the DPPH solution with 1 ml of each sample solution, which varies in concentration from 20 to 100 ppm. Adjust the volume to 10 ml by adding ethanol. Concurrently, generate a control solution by combining the DPPH solution with the solvent alone.All of these mixes should be incubated in darkness at room temperature for a duration of 30 minutes. During this period, the DPPH radical has a reaction with the antioxidants present in the samples, resulting in a noticeable alteration in color from purple to yellow. After the incubation period, the absorbance of each solution should be measured at about 517 nm using a spectrophotometer. The quantification of the residual concentration of DPPH serves as an indicator of the antioxidant activity of the samples, with a higher concentration of DPPH corresponding to a lower antioxidant activity.

In order to conduct a comprehensive analysis of the data, it is necessary to estimate the percentage of scavenging activity by comparing the absorbance values of the sample with those of the control.The formula for calculating percentage inhibition is expressed as follows: [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] × 100.

The construction of a graph depicting the percentage of scavenging activity as a function of sample concentration facilitates the characterization of the antioxidant activity profile. A positive correlation exists between the percentage of inhibition and the potency of the antioxidant activity exhibited by the sample. The IC50 value is determined by employing the fitted line equation, $y = mx + c$, where IC50 = (50 - c) / m(17) (18) (19)

In-vitro evaluation of Proangiogenic activity of the phytosomes by CAM assay: The chicken chorioallantoic membrane (CAM) assay is a standard assay used to evaluate the angiogenic potential of biomaterials. Angiogenesis is the formation of new blood capillaries from existing blood vessels for supplying nutrients to cells that are distant from existing blood vessels. Angiogenesis is a complex process that is mediated by the endothelial cells that line blood vessels. Angiogenesis is a regulated process involving a number of stimulators such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), angiopoietins, activators of integrins, and inhibitors such as thrombospondin, angiostatin, and endostatin. The extract and prepared phytosomal formulation were used for the comparative analysis of the proangiogenic activity.

Method: The chick chorio-allantoic membrane (CAM) experiment, as described by Ribatti et al. (1997), was employed to evaluate the impact of test substances on angiogenesis. The subject in question is of a mere 12 days in age. Fertilized chicken eggs were procured from a hatchery located in Nallakunta, Hyderabad. The eggs underwent a cleaning process using a solution of 70% ethanol. Subsequently, they were placed in an incubator set at a constant humidity level and a temperature of 37°C. During the treatment session, a little aperture was created at the constricted extremity, and a volume of 2-3 ml of albumin was extracted using an 18-gauge hypodermic needle. The clear tape was used to seal the window, after which it was incubated once again. Following a 24-hour incubation period, a tiny square aperture was created in the shell, allowing for the placement of a sterile gel foam piece of 3mm×3mm×1mm onto the membrane surface. The control group of vehicles was treated with sterile normal PBS (pH-7.0), whereas the test groups were treated with the necessary dose of test compounds that exhibited significant cytotoxicity against breast cancer. The eggs were subsequently placed back into the incubator and remained undisturbed for a period of 24 hours. Following the incubation time, the eggs were extracted from the incubator, and photographs of each egg treated with the chorioallantoic membrane (CAM) were acquired and subjected to analysis for the presence of blood vessels. The quantification of vascular branch points inside a square region, equivalent in area to each sponge, was performed. The analysis involved examining the results obtained from two preparations of the chorioallantoic membrane (CAM) for each treatment group. The angiogenesis index is determined by calculating the average number of new branch points in each set of data(20) (21) (22) (23).

3.Result:

TLC Analysis: Thin layer chromatography was carried out on a processed silica gel 60 F254 plate. Quercetin is used as a reference for hydrolyzed arjuna extract. The mobile phase was optimized using different solvent systems. The final mobile phase was Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2). The plates were examined in ultraviolet light at 254 nm. The RF value was found to be 0.61 and 0.62 for standard and extract respectively. Fig.2

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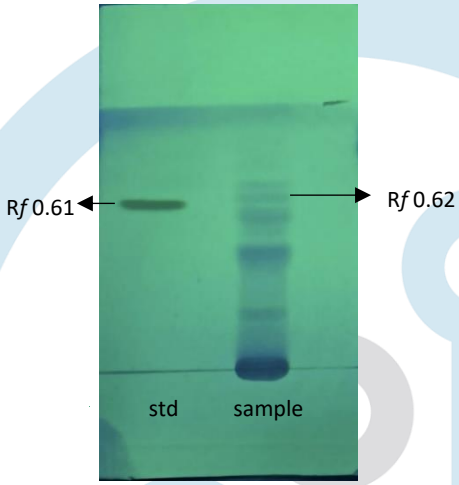

Fig 2.TLC

Determination of λ max: The concentration of 10mg/100ml *Terminalia arjuna* ethanolic extract in 5.5 phosphate buffer was found to be 254 nm. A graph is shown in Fig.3

Preparation of standard calibration curve of *Terminalia arjuna* extract: The UV absorbance of *Terminalia arjuna* extract standard solution in the range of 10-80 ppm phosphate buffer showed linearity at λ max 254 nm. The linearity was plotted against concentration with an R2 value of 0.9973 for phosphate buffer PH 5.5 as shown in Fig.4

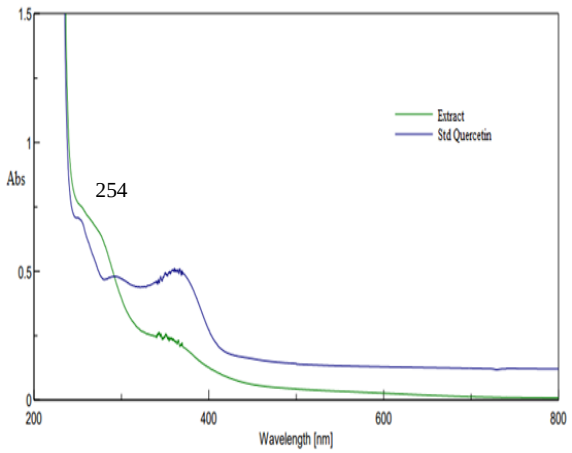

Fig 3: UV scanned for extract and std quercetin

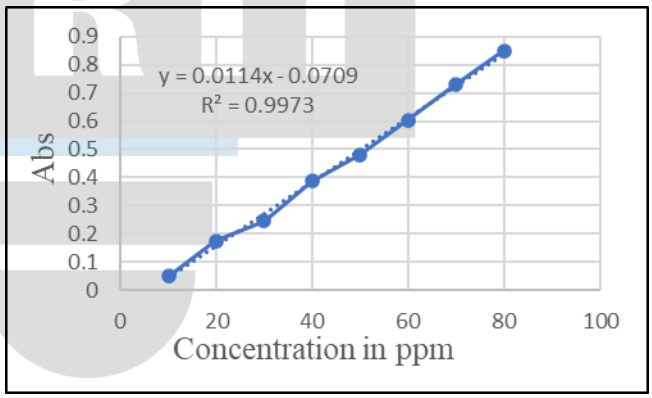

Fig 4: Calibration curve

Preparation of Phytosomes containing *Terminalia arjuna* ethanolic extract: Phytosomes of *Terminalia arjuna* extract were successfully prepared by using the solvent injection method in the ratios of lipid and extract 1:1,1:0.3,5:1,6:1,7:1. The prepared phyosomes batches kept at a room temperature in plastic container bottles to observed for the settlement of particles. From the below batches, the F7 batch shows down the suspended particle for more than a week. By using that ratio, we applied a QbD Approach of central composite design 2^3 factorial.

Optimization by QbD approach

Table no:1 Formulation table obtained from Qbd approach

Run	Factor 1A: Conc of lipid (mg)	Factor 2 B: Conc of cholesterol (mg)	Factor 3 C: Stirring time (hrs.)	Response 1 Entrapment efficiency(%)	Response 2 Particle size (nm)
1	1.5	0.9	3	89	279
2	3	0.4	3	86	276
3	1.5	0.4	1	84	274
4	3	0.9	1	88	278
5	3	0.4	1	82	272
6	1.5	0.9	1	85	275
7	3	0.9	3	84	275
8	1.5	0.4	3	82	274

Response surface analysis of entrapment efficiency:

The effects of CMAs and CPPs on the entrapment efficiency of PNPs can be observed in 3D response surface graphs and 2D contour plots. It was observed that as the stirring speed increased the entrapment efficiency also increased as seen in Fig 5. Entrapment efficiency decreased as the lipid: Cholesterol ratio decreased.

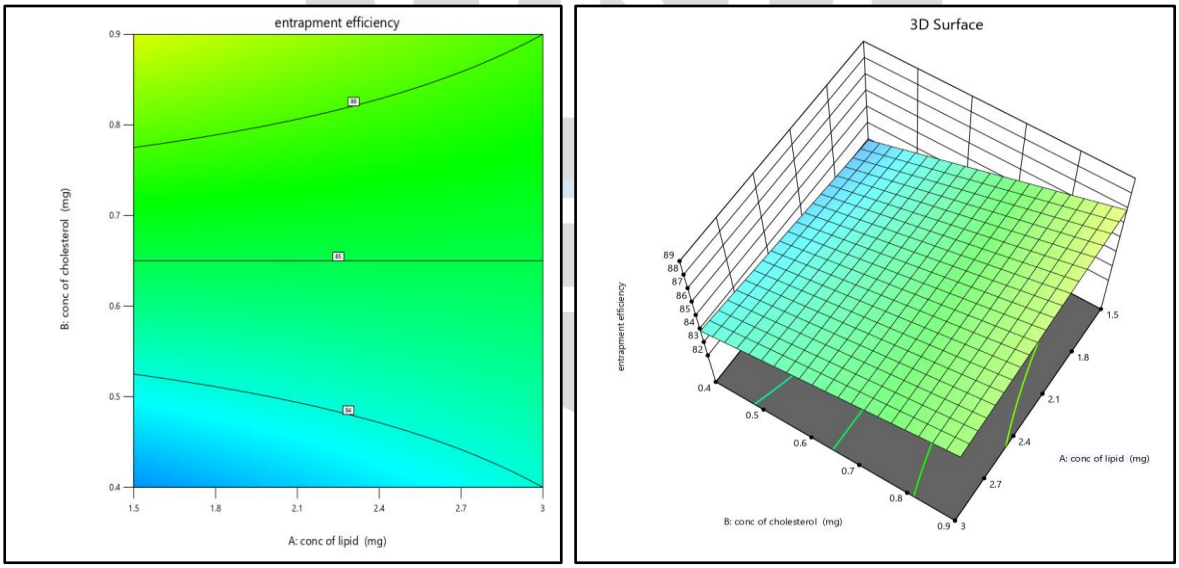

Fig 5. 2D contour plots and 3D response surface showing the effect of different CMAs and CPPs on entrapment efficiency

Response surface analysis of particle size:

The effects of CMAs and CPPs on the particle size of PNPs can be observed in 3D response surface graphs and 2D contour plots. It was observed that as cholesterol concentration increased the particle size increased as seen in Fig 6. Also, as the concentration of soya lecithin increased the particles formed were of smaller size. stirring speed was directly proportional to the particle size in the formulation. The concentration of soya lecithin and cholesterol affects the particle size of phytosome.

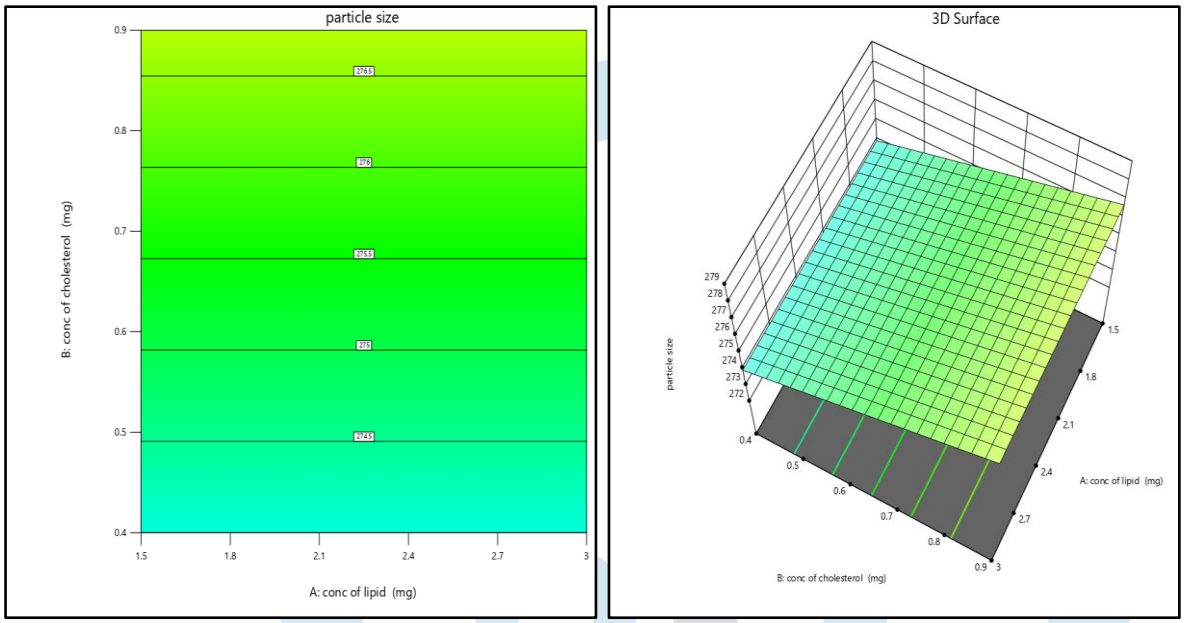

Fig 6. 2D contour plots and 3D response surface showing the effect of different CMAs and CPPs on particle size

Characterization of phytosome: The study determined that the mean particle size of phytosomes containing Terminala arjuna ethanolic extract was measured to be 272 nm, while the Zeta potential value was observed to be -16 mv. These findings suggest that the formulation exhibits favourable stability. The findings were visually depicted in Fig No 7&8.

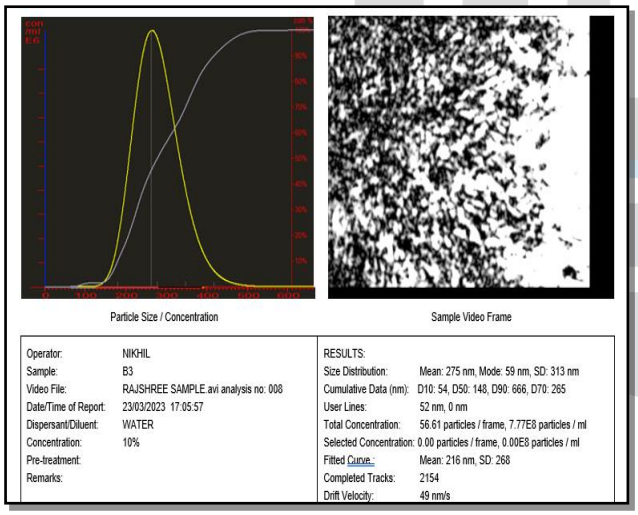

Fig 7: Particle Size

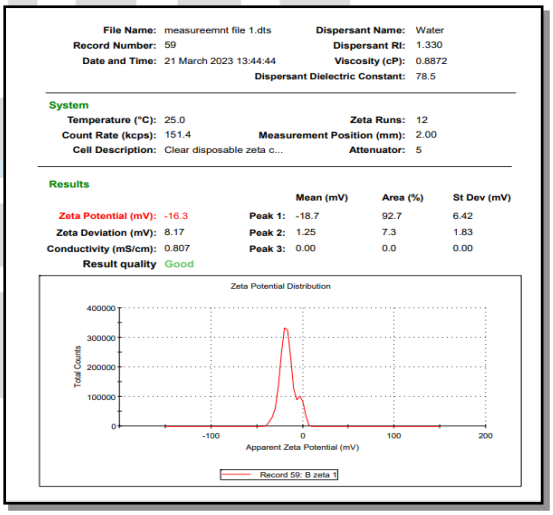

Fig 8: Zeta Potential

DSC: The differential scanning calorimetry (DSC) thermograms of the ethanolic extract of *Terminalia arjuna*, soya lecithin, and the phytosomes produced from the ethanolic extract of *Terminalia arjuna* were demonstrated. The ethanolic extract of *Terminalia arjuna* exhibited two distinct peaks, with an endothermic peak observed at around 101.15 °C and another peak observed at 105.00 °C. This phenomenon is commonly observed in second-order phase transitions, specifically in the context of glass transition at a temperature of 18 degrees. Soya lecithin has two distinct endothermic peaks at temperatures of 77.38 °C and 81.91 °C, respectively. The phytosome formulation exhibits two distinct peaks at temperatures of 95.69°C and 109.88°C. The observed phenomenon might perhaps be attributed to the liquefaction of the lipid component and its subsequent interaction with the ethanolic extract. This observation indicates that the ethanolic extract has been encapsulated within the lipid vesicles in Fig 9.

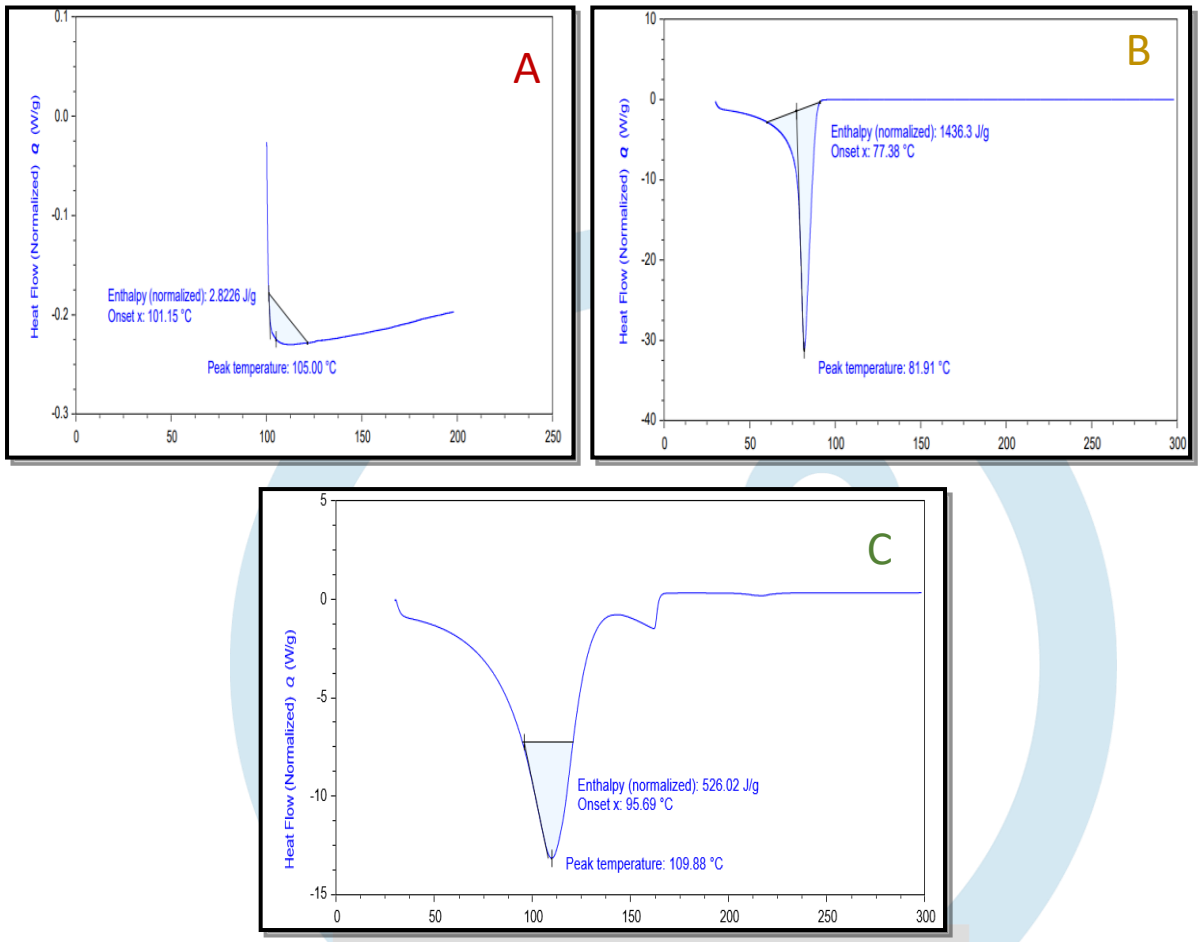

Fig 9: DSC thermogram of A: *Terminalia arjuna* ethanolic extract, B: Soya lecithin, C: Phytosomal formulation of *Terminalia arjuna*

FTIR: The Fourier Transform Infrared (FTIR) spectrum of the ethanolic extract of Terminalia arjuna, as well as the spectra of soya lecithin, cholesterol, and the phytosomes that were synthesized, are depicted in Figure 10. FTIR studies were carried out on Ext, lipid, and in the phytosomes to evaluate the existence of any interactions between bioactive compounds in Ext and lipid. The above fig shows the FTIR spectra of Ext, lipids, and phytosome formulations. The FTIR spectrum of lipids exhibited the characteristic signals at 2921 cm−1 and 2853 cm−1, corresponding to the C-H stretching in the chain of long fatty acids. Additional signals were also observed at 1733 cm−1 (C-O stretching in the fatty acid ester), 1252 cm−1 (P-O stretching), 1093 cm−1 (P–O–C stretching), and968 cm−1 [$-N^{+}(CH2)^{3}$]. In the Ext FTIR spectrum, the major peak was observed at 1694 cm−1 due to the C-O stretching of Quercetin contained in Ext. The peak at 1725 cm−1 was corresponding to carboxylic acid from Quercetin. Due to the rich amount of phenolic compound in Ext, the peak between 1200 and 1400 cm−1 were observed, corresponding to –OH phenolic bending. In the phytosomal formulation, it was found that the peaks of –OH and C-O of Ext were shifted to a higher wave number. Furthermore, the peak of P-O of lipid was broadened. In addition, the broadening of the characteristic phenolic (-OH) band at 3500 cm−1 was observed, which could be potentially caused by the formation of H bonding.

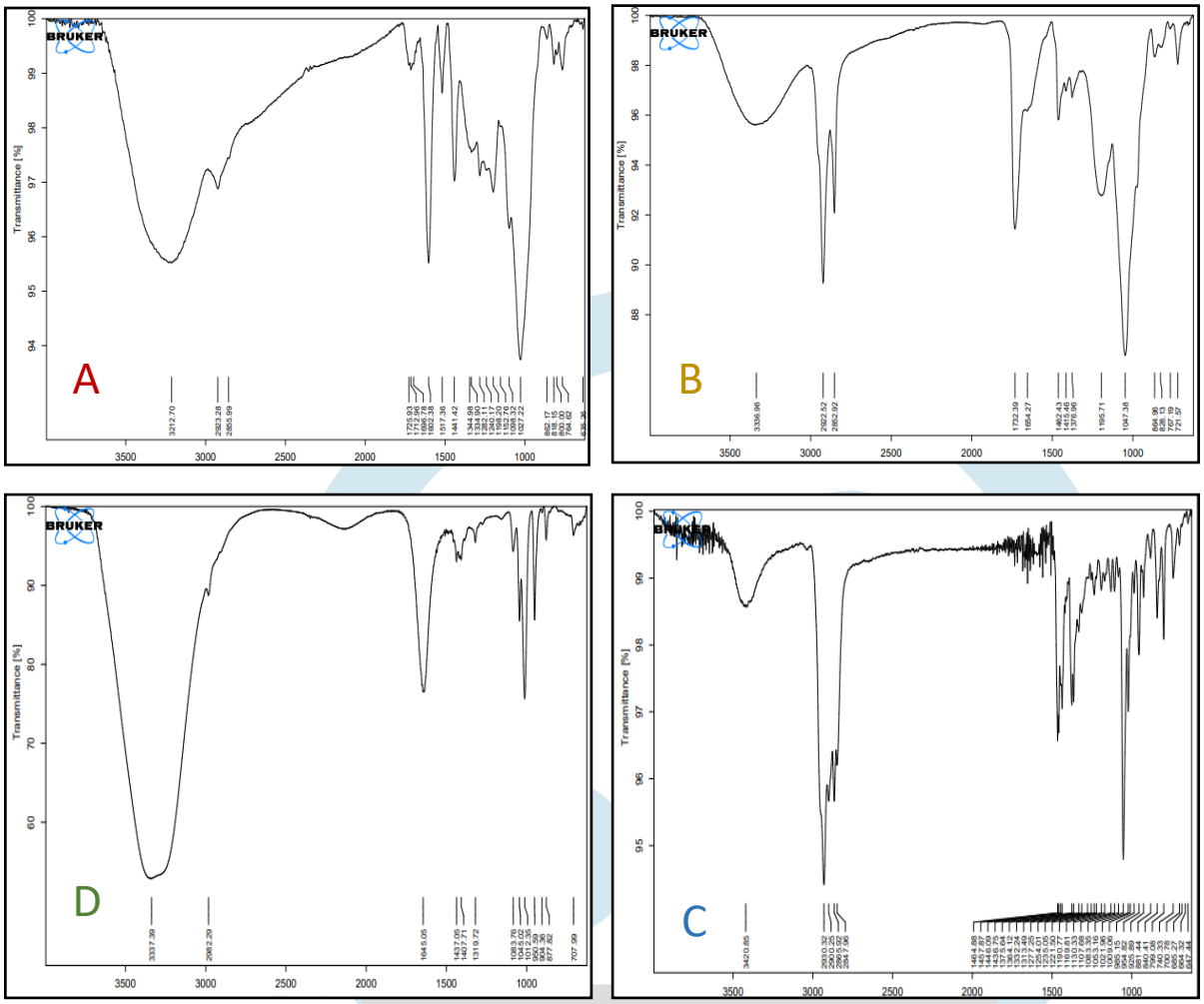

Fig 10: FTIR Graph of A: *Terminalia arjuna* ethanolic extract B: Soya lecithin C: Cholesterol D: Phytosomal formulation of ethanolic extract

Stability studies: The ethanolic extract of Terminalia arjuna was used to prepare phytosomal formulations (F7), which were then kept at both room temperature and refrigerated temperature for a period of 4 months. The entrapment effectiveness of the formulations was afterward measured. At a controlled temperature of 25 ºC ± 2ºC, it was observed that there was no statistically significant alteration in the entrapment effectiveness of the phytosomal formulation. See table no.2. A reduction in entrapment efficiency occurred at a temperature of 4°C ± 2°C, following a period of one month. See table no.3.

Table No.2 Phytosomes stability at room temperature

Time points	Physical appearance	Particle Size	Entrapment Efficiency
0 months	Clear solution	272nm	84%
1 months		280nm	83.58%
2 months		285nm	81.70%
4 months		292nm	80.12%

Table No.3 Phytosomes stability at refrigerated conditions

Time points	Physical appearance	Particle Size	Entrapment Efficiency
0 months	Clear solution	272 nm	84%
1 months		320 nm	80.38%
2 months	Turbid solution & Sediment the particle	405 nm	76.89%
4 months		720 nm	70.24%

In-vitro release study: The cumulative release percentage of an optimized phytosomal batch of an ethanolic extract derived from Terminalia Arjuna was determined to be 78.64% during a 12-hour release assay, as seen in. The ethanolic extract of Terminalia arjuna exhibited the highest release, reaching 79.98% within the first two hours. Subsequently, the release rate decreased. The Phytosomal batch exhibits a sustained release profile as a result of the encapsulating of the extract using a combination of soya lecithin and cholesterol. see fig no 11.

In-vitro antioxidant assay: The IC50 value is the minimum inhibitory concentration at which the sample scavenges the 50 % free radical. The minimum inhibitory concentration of ascorbic acid, ethanolic extract of Terminalia arjuna, and its phytosomal formulation was found to be 116.8%,86.53%, and 60.83% respectively. Hence proved that the formulation shows maximum antioxidant activity imparting good cardiovascular health benefits.see fig no.12

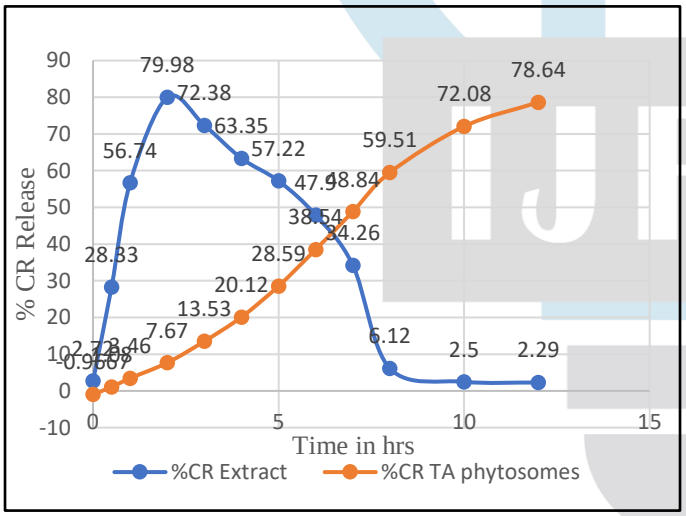

Fig:11 *In Vitro* Release from *Terminalia arjuna* ethanolic extract and its phytosomal formulation

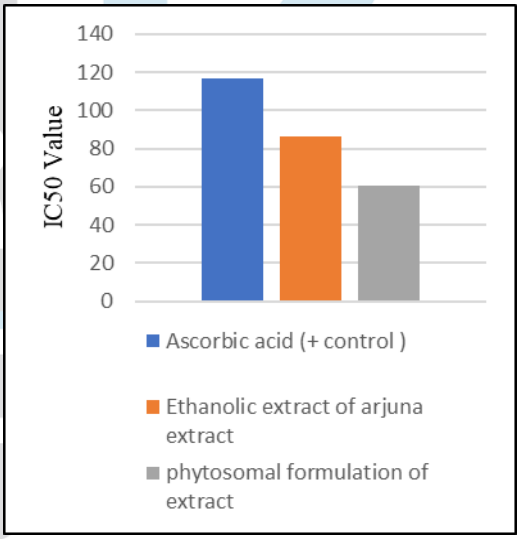

Fig:12 *In vitro* Antioxidant activity of ethanolic extract and its phytosomal formulation

Kinetic model

Terminalia arjuna extract release from phytosomal lipid bilayer (points representing average values) fitted to the kinetic models (lines). Each kinetic model was fitted with the First 78% release: The cumulative% drug release versus time for a zero-order kinetic model, the cumulative% drug release versus time for a first-order kinetic model, the cumulative% drug release versus square root of time for a simplified Higuchi model, and the cumulative% drug release versus log of time for a Korsmeyer-Peppas model.
With R2 > 0.98, the zero-order kinetic and Korsmeyer-Peppas models had a significant correlation. Refer table no 4 and fig no.13.

Table No 4: Kinetic Model Table

Kinetic model	R^2	K/N
Zero-order kinetic	0.98	0.100
First order kinetic	0.61	0.045
Higuchi Kinetic model	0.88	11.40
Kors Meyer Peppas model	0.99	N=0.49

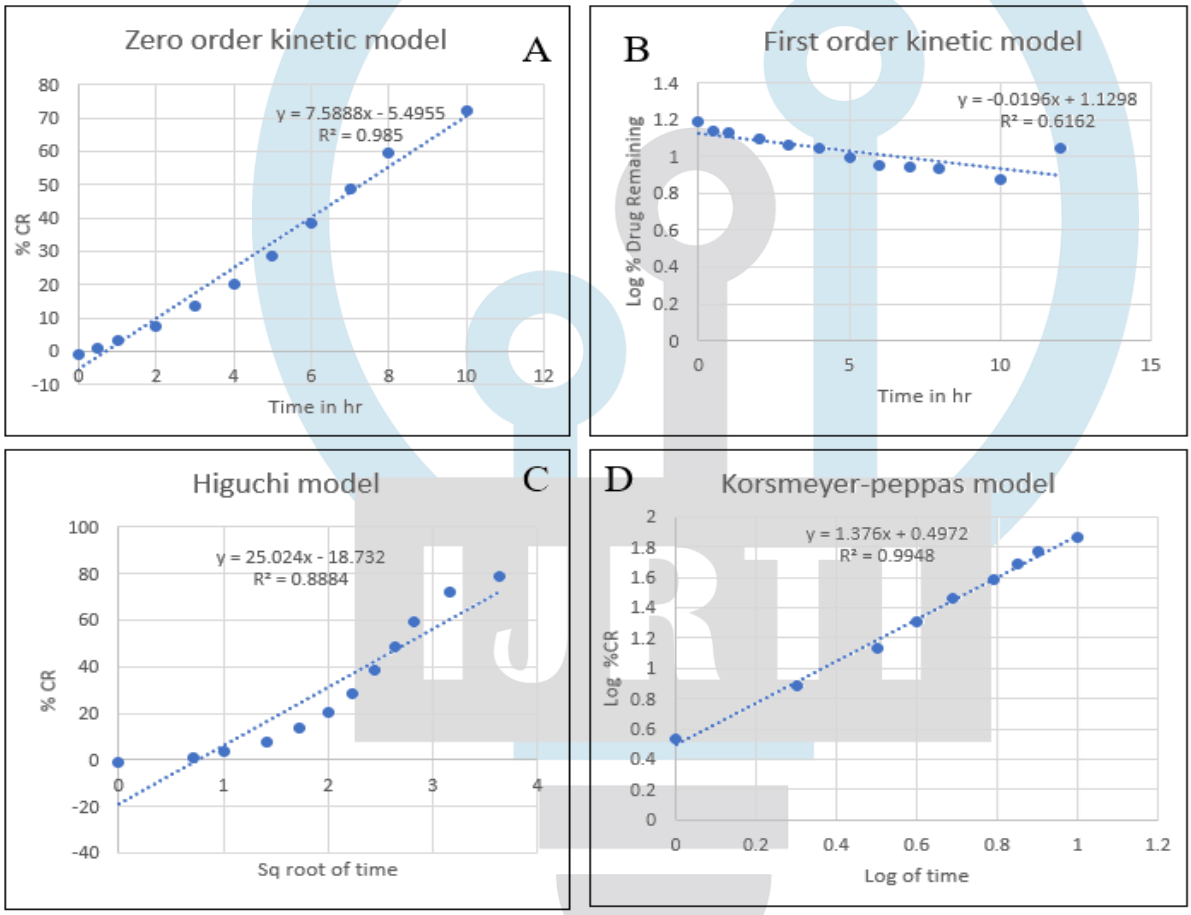

Fig 13. Kinetic models A: Zero-order kinetic model B: First-order kinetic model C: Higuchi model D: Korsmeyer-peppas model

In-vitro evaluation of Proangiogenic activity of the phytosomes by CAM assay:

The study revealed that test samples RP2 i.e., phytosomal formulation of *Terminalia arjuna* extract exhibited notable angiogenic activity in the Chick Chorioallantois membrane (CAM) assay, with increased formation of new blood vessels i.e 51±4.24.Ref table no 5. This angiogenic response holds significant therapeutic promise as it plays a pivotal role in various physiological processes, including wound healing and tissue regeneration. Enhanced angiogenesis can ensure a more efficient supply of oxygen and nutrients to cells, aiding in the recovery process. Additionally, it suggests potential applications for these compounds in treating conditions characterized by impaired angiogenesis, such as cardiovascular diseases and chronic wounds, where promoting blood vessel growth can facilitate healing and tissue repair, ultimately improving patient outcomes. See fig no 14.

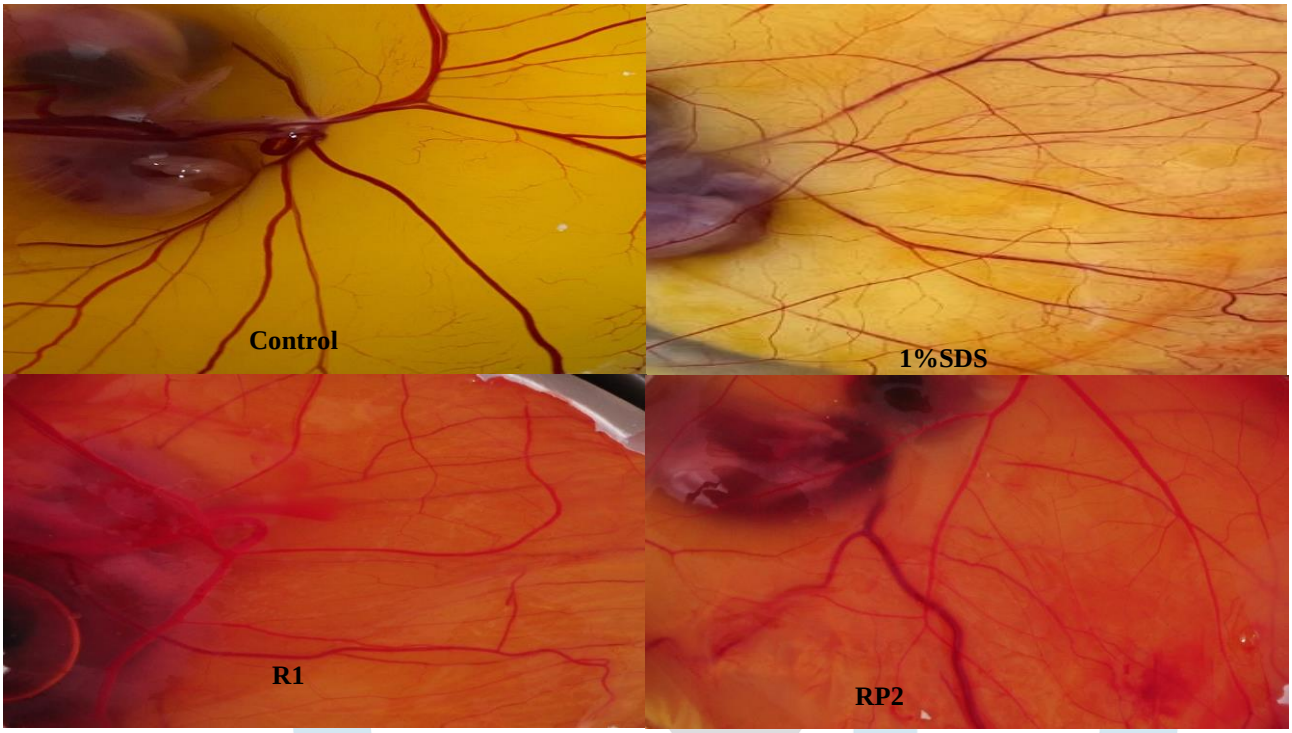

Fig 14: Chorioallantoic membrane (CAM) assay. Control, 1% SDS, R1, and RP2 with 100ul respectively. It was clear that the formation of new blood vessels was moderately appeared in the RP2 as compared to the vehicle-treated CAM.

Table No 5: In-Vitro data of proangiogenic; No of branching vessels

Sr. No	Condition	No of branching vessels ± SD
1	Control (PBS)	21.5±4.95
2	Std control-1% SDS	49±4.24
3	R1-100ul	41±2.83
5	RP2-100ul	51±4.24

4.Discussion: Cardiovascular diseases, including heart stroke, are major causes of mortality worldwide. When the heart or other parts of the cardiovascular system are compromised, it can lead to reduced blood flow and oxygen supply to vital organs, which can result in severe health consequences. One potential therapeutic approach for treating cardiovascular diseases is the formation of new blood vessels, a process known as angiogenesis. In the context of cardiovascular diseases, stimulating angiogenesis can help improve blood flow and restore tissue perfusion to damaged areas. This, in turn, may enhance tissue repair, alleviate ischemic conditions, and potentially prevent further cardiovascular complications.

Arjuna extract has exhibited pro-angiogenic activity. An increase in the formation of the blood vessels was observed when treated with arjuna bark extract; however, the formation of the blood vessels was more in phytosomes treated group, and this can be attributed to more bioavailability of phytosomes as compared to herbal extract. Hence, there is probably a gradual and sustained release of phytosomes ensuring stability and optimizing the therapeutic effects of an encapsulated therapeutic compound.

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6.Conflict of interest: Conflict of interest declared none.

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