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FORMULATION AND EVALUATION OF PHYTOCONSTITUENTS CREAM FOR THE TREATMENT OF VARICOSE VEINS

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ABSTRACT

Phytoconstituents rutin and quercetin are used to assist the treatment of venous disorder like varicose veins and hemorroids, which reduces the capillary permeability and boost the integrity of vessels. There are certain limitations to the use of these phytoconstituents in the pharmaceutical formulations because of their physical properties like limited aqueous solubility, poor bioavailability and high oral dose. Therefore in the present research work cream of rutin and quercetin were developed for improving solubility and bioavailability using topical route.A mixture of rutin and quercetin was formulated in o/w cream. The cream was optimized to achieve good spreadability and highest drug diffusion using a two factor glyceryl caprylate (GC) and triethanolamine (TEA) three level (spreadability, viscosity and drug diffusion) factorial design. The cream was then characterized for physicochemical parameters. *In-vitro* diffusion and *ex-vivo* permeation studies were performed to estimate the diffusion of the drugs from the prepared formulation.It was observed from *ex-vivo* permeation studies that the flux for optimized cream was found to be 0.1898 and 0.3481mg/hr/cm^{2}respectively as compared to the saturated solution of pure rutin (0.1035mg/hr/cm^2) and quercetin (0.1264mg/hr/cm^2).The formulation was optimized and had viscosity 8190Cps, spreadability 56.4g and *in-vitro* drug diffusion flux as 0.2053 mg/hr/cm^2 for rutin and 0.1621mg/hr/cm^2 for quercetin respectively. Thus, it can be concluded that the components are contributing to providethe phytoconstituents at the site of action by topical cream optimized for the spreadability, viscosity and drug diffusion which control the release and entry of actives through skin barrier.

KEYWORDS: Rutin, Quercetin, Venous disease, Topical cream, *ex-vivo* permeation.

INTRODUCTION

Varicose Veins is a disorder of the veins (especially of legs) wherein they get affected due to the backward flow and turbulence in the circulation of the blood.[1] The veins get perverted and become enlarged due toedema. The exact pathophysiology is debated, but it involves a genetic predisposition, incompetent valves, weakened vascular walls, and increased intravenous pressure. A heavy, achy feeling, itching or burning and worsening with prolonged standing are all symptoms of varicose veins. Potential complications include infection, leg ulcers, stasis changes, and thrombosis.[2] Some treatment options are external compression, loosening of restrictive clothing, medical therapy, modification of cardiovascular risk factors, reduction of peripheral edema, and weight loss. More aggressive treatments include external laser treatment, injection sclerotherapy, endovenous interventions and surgery.[3]

Choice of therapy is affected by symptoms, patient preference, cost, potential for iatrogenic complications and available medical resources. After review [4] regarding pathogenesis as well as different traditional and alternative therapy for treating varicose veins, we have identified phytoconstituents rutin and quercetin which are reported to have good effect on the veins helping them in better functioning. Rutin through its free radical activity is able protect these walls and it inhibit the PAF (platelet activating factor) and thromboxane A2 thus diminishing capillary permeability.[5,6] Quercetin dramatically stabilize small blood vessels relating to the veins, helping reduce fluid retention and specifically boost integrity of vessels.[7] Presently these phytoconstituents are administered orally in supplements form with very high doses and which show poor bioavailability.

Therefore the present work attempts to develop a topical cream containing rutin and quercetin using principles of formulation design and design of experiment strategy to get a formulation with desired performance attributes.

MATERIALS AND METHOD

Materials

Rutin was acquired from LobaChemie Pvt. Ltd., (Mumbai, India) and Quercetin was obtained from Green Heaven India Pvt. Ltd., (Nagpur, India).

All other ingredients used in the formulation of cream were purchased from local market and were of extra pure grade.

Development and formulation of cream

Drug Excipient Compatibility[8]

Compatibility of rutin and quercetin with formulation excipients such as stearic acid, glyceryl caprylate, glyceryl monostearate, isopropyl myristate, propylene glycol and glycerine was determined by keeping them together in hermetically sealed glass vials at 40^0C for two weeks. Subsequently FTIR (Jasco FTIR 4100) analysis was performed on individual components and drug excipient mixtures between 400 cm -1 to 4000 cm-1 to check for any changes in the functional groups.

Determination of saturationsolubility of phytoconstituents[9]

The solubility of rutin and quercetin in glyceryl caprylate, mineral oil and water was determined. Excess of rutin and quercetin were added separately to5 mL each of GC, mineral oil and water. All the six dispersions were kept for shaking in orbital shaker (Model: CIS-24 BL) for 48 h. Clear supernatant was taken and diluted to suitably with ethanol and samples were analyzed by UV spectrophotometer at the wavelength 257nm and 372nm respectively.

Selection of cream formula

Cream formula was selected from literature[10] as presented in Table 1.

Table 1: Formula for cream.

Sr.no	Ingredients	Quantities (%)
1.	Rutin	1.0
2.	Quercetin	1.0
3.	Stearic acid	20.0
4.	Glycerylcaprylate (GC)	20 .0-30.0
5.	Lanolin	0.8
6.	Glycerylmonosterate	3.0
7.	Isopropyl myristate	2.0
8.	Propylene glycol	4.0
9.	Glycerine	4.0
10.	Triethanolamine (TEA)	2.0-4.0
11.	Water	q.s. 100
12.	Perfume	q.s

Design of experiment

Experimental Factorial design (2 factors, 3 levels) was chosen to derive formula which provided optimum spreadability, viscosity and drug diffusion. The factor combinations, levels and responses are shown in the table below (table 2).

Table 2: Factors and responses chosen for the design.

Variables		Levels		
A	Glycerylcaprylate	+1	0	-1
		20ml	25ml	30ml
B	Triethanolamine	2ml	3ml	4ml
Responses		**Goals**	**Acceptance range**	
X_1	Spreadabilty	Optimum	<60 g	
X_2	Viscosity	Optimum	<700 Cp	
X_3	Drug diffusion	Maximum	91-101%	

Preparation of phytoconstituents cream

The oil phase was prepared by melting the waxesat 70^0 C and quercetin was added with constant stirring. The aqueous phasewas prepared by dissolving the water soluble ingredients and rutin in distilled water and was warmed to 70^0C. The aqueous phase was slowly added to the oil phase with constant stirring at 100rpm by using propeller(Remi motor RQT-127 HP1/8) until the temperature dropped to 40^0C .The emulsion was cooled to room temperature to form a semisolid cream.

Evaluation of phytoconstituents cream[11]

(i) Spreadability

Hardness of formulation was determined using a texture analyzer (Brookfield CT-3) cream (20 gm) was filled in conical probe (up to plane surface of top) and hardness was measured. This apparatus shows hardness into the comparison of spreadablity and adhesive force.

(ii) Viscosity

The viscosity of the formulation was determined using viscometer (Brookfield digital viscometer RVDV Pro) equipped with ULE adapter. The spindle (S-06) was rotated at 50 rpm. Samples of the cream were allowed to settle over 30 min at the temperature (25 ± 10^0C) before the measurements were taken. Viscosity was reported in (cP).

(iii) Analysis of drug content by first derivative spectroscopy[12]

Accurately weighed 1g of formulated cream sample was transferred to 10 ml volumetric flask and volume was adjusted using ethanol. The resulting solution was filtered using 0.45 µm

filter and suitably diluted with ethanol. This solution was then analyzed at the wavelength 257 and 372 nm using UV Spectrophotometer (JascoV730) and the above spectra was further converted to first derivative spectroscopy and the absorbance of rutin was recorded at zero crossing point of quercetin (372nm) and the absorbance of quercetin was recorded at the zero crossing point of rutin (257nm) to determine the content of rutin and quercetin in the formulation respectively.

(IV) *In-vitro* drug diffusion studies[13]

The release of drug from the cream was determined using Franz diffusion cell apparatus for 6h.The receptor medium was hydroalcoholic solution containing phosphate buffer and ethanolin the ratio (7:3) at pH 7.4, maintained at 37^0C. The membrane filter used was (cellulose acetate)membrane pore size 0.45µ and soaked in hydroalcoholic solution pH 7.4 for 1h.The membrane was mounted between the donor and receptor compartment. The cream (300mg) was placed on receptor compartment and both the compartments were clamped together. The hydroalcoholic solution having pH 7.4 in the receptor compartment (8 ml) was stirred using magnetic stirrer 60 rpm. Aliquots of 1 mlwere withdrawn after each interval and the same amount of solution was replaced with the fresh hydroalcoholic solution pH 7.4. Collected samples were filtered through 0.45µm filter. Rutin and quercetin were quantified by using HPLC at 255nm.[14] Saturated solution of pure rutin and quercetin in pH 7.4 phosphate buffer were used as standards since topical marketed formulation of rutin and quercetin was not available. The amount drug release was calculated.

***(*v) *Ex-vivo* drug permeation studies**

For *exvivo* permeation studies abdominal shaved skin (3.14 cm2) of excised male Sprague-Dawley rat weighing 200-250 g was used and the procedure was followed as above for *invitro* diffusion. The amount drug release was calculated.

HPLC analysis of sample[14]

Rutin and quercetin concentrations in the diluted solutions were determined by HPLC using (HPLC system used was JASCO system equipped with model PU 2082 Plus pump, Rheodyne sample injection port (20 µl), JASCO UV 2075 Plus detector and Borwin chromatography software (version 1.5) with HiQSil C18 (250mm*4.6 mm, 5µm) column. The mobile phase was a mixture of acetonitrile: water (60:40); water pH adjusted to 3 by orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and pumped from the filter reservoir at a flow rate of 1 ml/min which yielded a column back

pressure of 110-120 bars. The run time was set at 10 min and the volume of injection was 20 µl. The column was equilibrated for at least 30 min with the mobile phase running through the system before injecting the drug solution. Then the samples withdrawn from Franz diffusion cell were diluted with the mobile phase filtered through sample filter and injected into the column. The eluent was monitored by isocratic elution at 255 nm.

(v)Skin irritancy test[15]

The animal study protocol was reviewed and approved by the Institutional Animal Ethical Committee, AISSMS College of Pharmacy (Regd no. 257/P0/ReBi/S/2000/CPCSEA, Dated-13/01/18), Savitribai Phule Pune University, Pune.The formulation containing the lowest effective strength was tested wistar rats. As follows: each rat was kept in different cage food was supplied during the test period. 24 hours prior to test the hair from the spine region was shaved. The test site was cleaned with surgical spirit then 1gm cream was applied to test area. The test site was observed for erythema and edema after 24hrs after application.

RESULTS AND DISCUSSION

Drug Excipient Compatibility

The FTIR spectrum of Rutin and Quercetin respectively was recorded and compared with that of the standards in the literature. The major peaks of rutin and quercetin could be seen as mentioned in the table-3 below while there was absence of any new peak other than that for rutin and quercetin peaks. From the spectra it can be concluded that there is absence of any well- defined interaction between the drugs and the excipients. Hence, drug – excipients compatibility was established.

Table 3: Interpretation of IR spectrum rutin and quercetin.

Interpretation of rutin		Interpretation of quercetin	
Wave number (cm^{-1})	**Functional group**	**Wave number (cm^{-1})**	**Functional group**
3439, 3321	-OH Stretching	3420, 3369	-OH Stretching
2924,2861	-CH_2 Stretching	2986	-CH_2 Stretching
2714	-CH bonding	2872	-CH stretching
1402	-C=O Vibration	1457	-(C=O) Stretch
1383	-C-OH Vibration	1362	-C-OH Vibration

Determination of saturationsolubility of phytoconstituents

Highest solubility of rutin was seen in water (30mg/ml) and that of quercetin was seen in glyceryl caprylate (40mg/ml). Hence, glyceryl caprylate was selected as internal phase. The solubility's are as follows in Fig. 1.

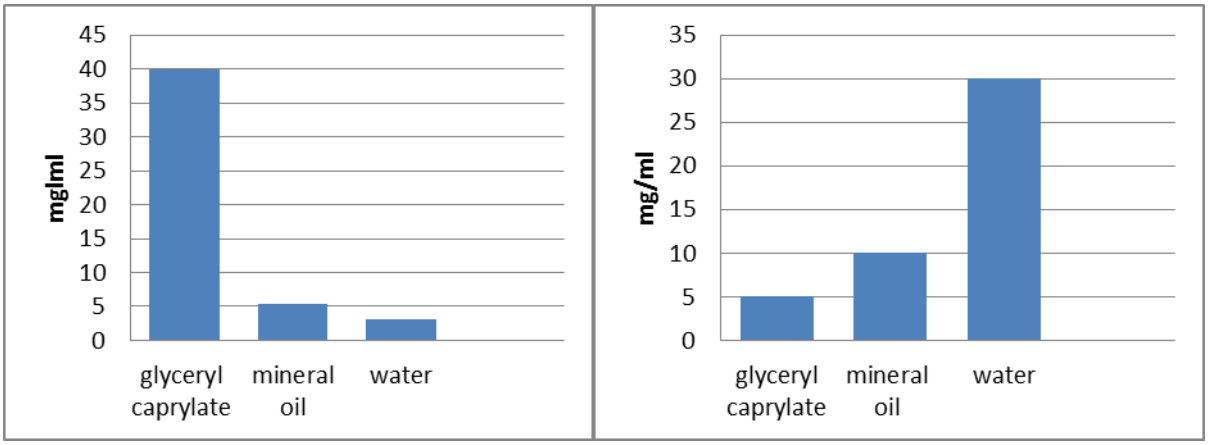

Fig 1: Solubility of rutin and quercetin indifferent solvents.

Design of experiment

Optimization of the formula (Table 1) was done using 2 factor 3 level factorial design using Design Expert 11.0 trial version. The responses such as spreadability, viscosity and drug diffusion were chosen for optimization as spreadability increases the ease on application, viscosity governs the drug release/ diffusion from the formulation and drug diffusion is an important criteria as it gives an idea whether the drug is able to reach the site of action or dermis of the skin. These factors depend mainly on quantity of oil phase and the alkalizing agent hence were selected for optimization using three levels give good idea about interaction between factors if any.

Evaluation of phytoconstituentcream

All the formulated formulations (B1-B9) were evaluated for spreadability, viscosity,drug content and drug diffusion (Table 4). The drug content of rutin and quercetin in the formulations varied between 24.04 - 104.98% and 26.30 – 96.50% respectively. The globule diameter was found in the range of 501 – 759.2 nm respectively.B3formulation shows highest drug diffusion of rutin and quercetin respectively.

Table 4: Evaluation of trial runs of factorial design.

Formulation code	Factor A: B GC:TEA (%)	Response1: spreadability (g)	Response 2: Viscosity (Cp)	Response 3: Drug diffusion 1 and 2 (flux)		Drug content(%)		Globule diameter (nm)
				Rutin	Quercetin	Rutin	Quercetin	
B1	20:2	81.8	8128	0.12	0.03	72.95	33.92	732.9
B2	25:2	63.7	8128	0.16	0.05	89.05	52.11	485
B3	30:2	56.4	8190	0.20	0.16	104.9	96.50	466.5
B4	20:3	63.54	7840	0.08	0.03	80.53	76.83	501
B5	25:3	76.9	7590	0.09	0.05	52.37	78.24	618
B6	30:3	74.9	8650	0.15	0.12	86.11	95.6	702
B7	20:4	28	9680	0.09	0.03	23.66	16.30	492.3
B8	25:4	42.6	10100	0.13	0.05	24.04	32.87	759.2
B9	30:4	55.7	10140	0.16	0.15	62.15	92.39	698

(1)Spreadability

Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application of skin. The therapeutic efficacy of a formulation also depends on its spreading value. The spreadability was found in the range of 28 - 81.8 g. The equation (1) for spreadability is as follows.

Spreadability = $72.45 + 2.28A - 12.60B + 13.28AB - 1.01A^2 - 17.08B^2$…….(1)

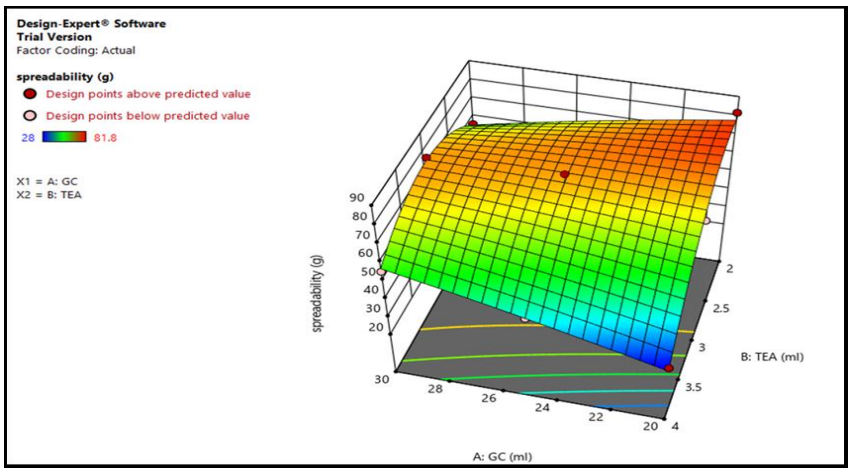

Fig. 2: Response surface depicting effect of GC and TEA on spreadibility.

The model terms for the cream spreadability (Fig. 2) were found to be significant with high value of R^2 0.9612 which indicates adequate to a quadratic model. Values of probability F was less than 0.5 which indicated that the model terms were significant. The predicated R-squared of 0.6161 is in reasonable agreement with the adjusted R-square 0.8965; i.e. the difference is more than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.1 it was found that the spreadability increases with increase in the concentration of GC as it lubricates the skin and make it easily spreadable

while as the concentration of TEA increases the spreadability decreases due to thickening of the cream beyond the limits that is (2-4%) due to which the spreadability is affected. This is due to higher quantity of soap which acts as emulsifier and reduces globule diameter.

(2) Viscosity

Viscosity is a very important which governs the release of the drug from the formulation.Viscosity was found in the range of 7590-10140 cps. The equation (2) for viscosity is as follows.

$$\text{Viscosity}=+7916.44+ 222.00A+ 912.33B+ 99.50AB+ 165.33A^2+ 1034.33B^2 \ldots.(2)$$

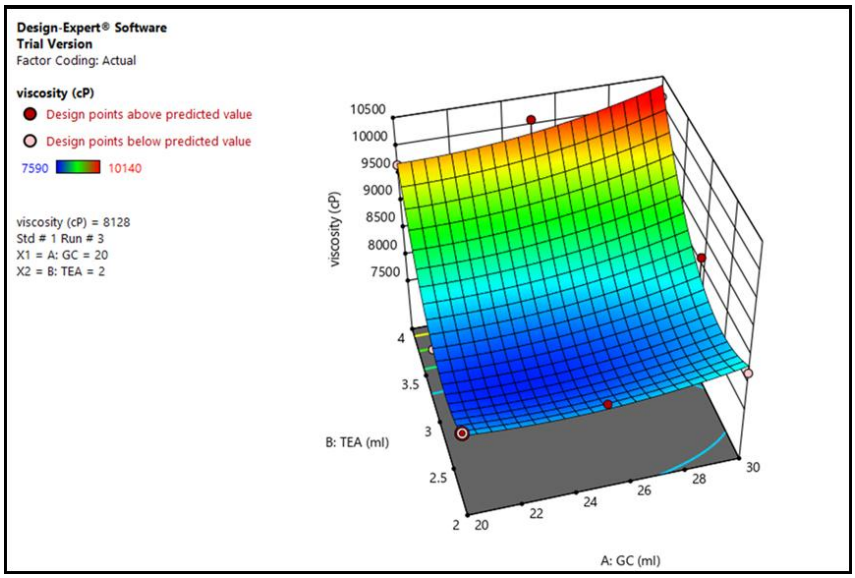

Fig. 3: Response surface depicting the effect of GC and TEA on Viscosity.

The model terms for the cream viscosity (Fig. 3) was found to be significant with high value of R2 0.9548 which indicates the adequate to a quadratic model. Values of probability F was less than 0.5 indicated that the model terms were significant. The predicted R-squared of 0.5930 is in reasonable agreement with the adjusted R-square 0.8794; i.e. the difference is more than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.2 it was found that the viscosity decreases with increase in the concentration of GC as it makes the cream more spreadable and less viscous while increasing TEA increases the viscosity due to uncoiling and physical entanglement of cars.

(3) Drug diffusion (1) of rutin

Drug diffusion is very important criteria which helps to understand whether the drug is diffused or the drug has reached to the dermis of the skin to act on the veins. The flux was

found to be in the range 0.09558- 0.2053 mg/hr/cm^2(fig 6). The equation for drug diffusion of rutin is as follows.

Drug diffusion 1= +0.1101 + 0.0360A - 0.0160B - 0.0031AB + 0.0060A^2 + 0.0338B^2…..(3)

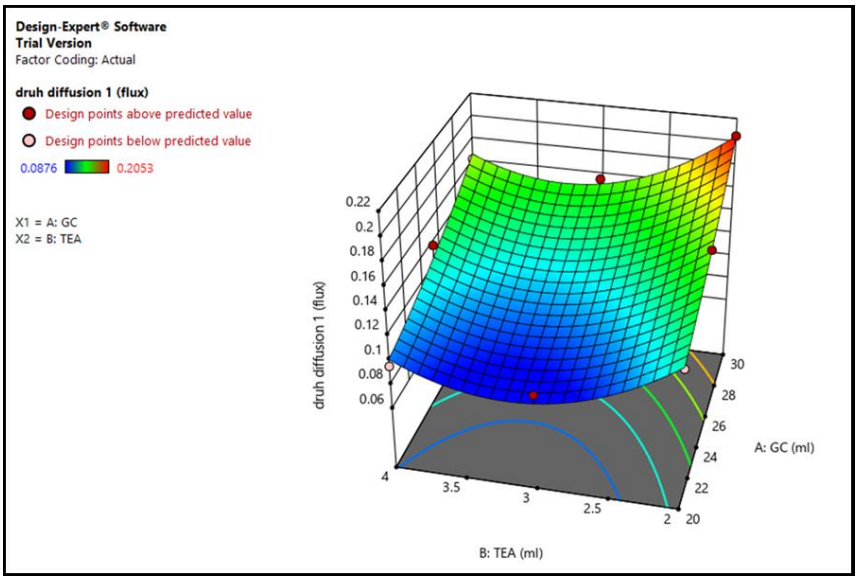

Fig. 4: Response surface depicting effect GC and TEA on drug diffusion of rutin.

The model terms for the drug diffusion (Fig. 4) of rutin was found to be significant with high value of R2 0.9701 which indicates the adequate to a quadratic model. Values of probability F was less than 0.5 indicated that the model terms were significant. The predicted R-squared of 0.7512 is in reasonable agreement with the adjusted R-square 0.9202; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.3 it was found that the drug diffusion of rutin decreases with increase in the concentration of TEA due to increase in viscosity of the formulation but at higher concentration, the diffusion increases which may be due to high surfactant concentration increasing permeability of skin.

(4) Drug diffusion (2) of quercetin

The flux of quercetin was found to be in the range of 0.034 – 0.1621 mg/hr/cm^2 (fig 7). The equation for drug diffusion of quercetin is as follows.

Drug diffusion of quercetin = + 0.0470 + 0.0560A - 0.0002B -0.0029AB + 0.0373A2 + 0.0108B2…..(4)

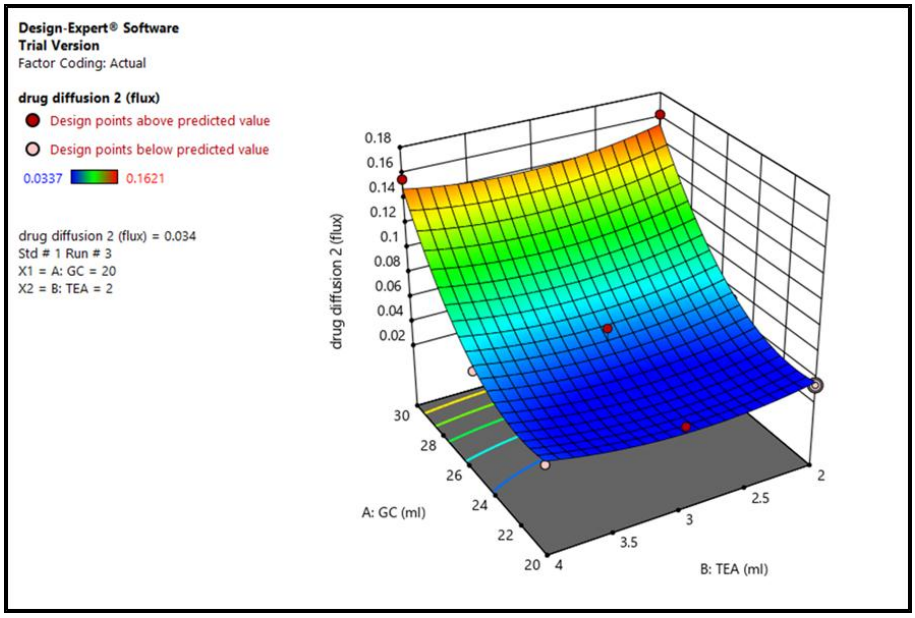

Fig. 5: Response surface depicting effect of GC and TEA on drug diffusion of quercetin.

The model terms for the cream drug diffusion of quercetin (Fig 5) was found to be significant with high value of R^2 0.9756 which indicates the adequate to a quadratic model. Values of probability F was less than 0.5 indicated that the model terms were significant. The predicted R-squared of 0.7498 is in reasonable agreement with the adjusted R-square 0.9348; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.4 it was found that glyceryl caprylate has little effect but increase in triethanolamine increases the flux as concentration of soap triethanolamine stearate is higher leading to small globule diameter and increases penetration.

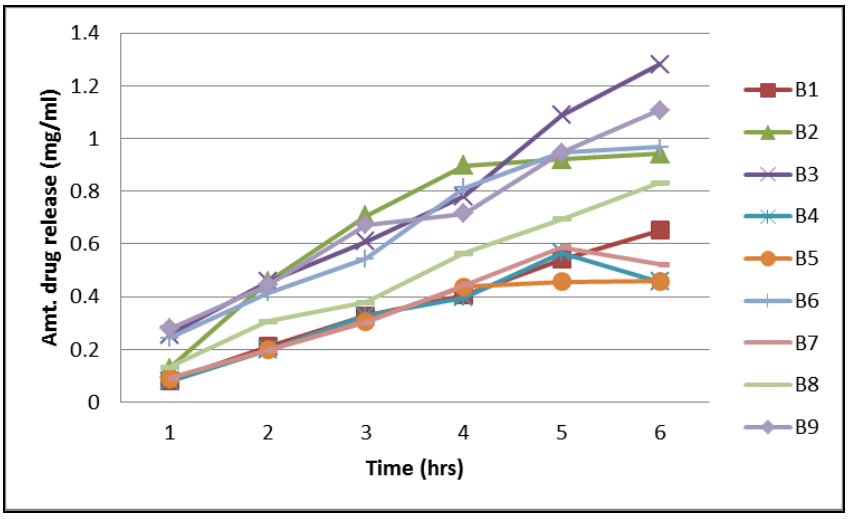

Fig. 6: Amount *in vitro* drug release (rutin) from optimization batches.

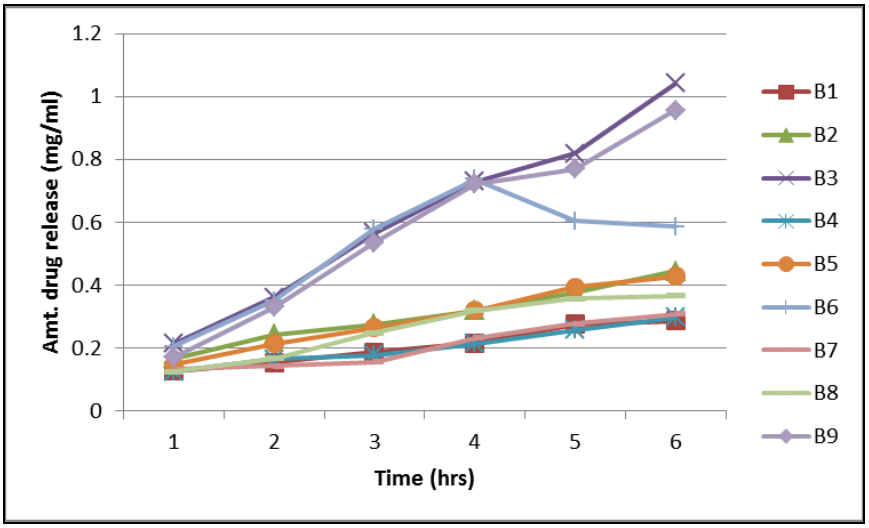

Fig. 7: Amount *in vitro* drug release (rutin) from optimization batches.

Fig. 6 and Fig. 7 demonstrate the data from *invitro* drug diffusion test in comparing the drug release from a formulation to another, it was observed that formulation B3 showed better flux (0.2053 and 0.1621 mg/hr/cm^2) for rutin and quercetin respectively due to presence of excipients like glyceryl caprylate with better penetration and absorption and optimum concentration of surfactant.

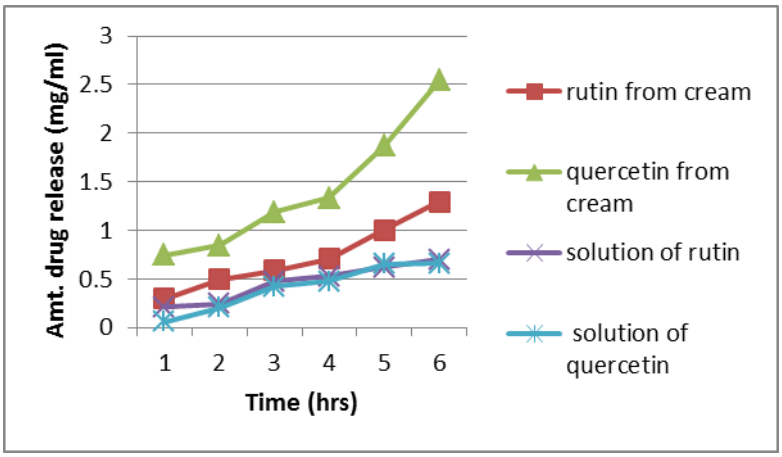

Fig. 8: Amount *Ex-vivo* drug release from optimized batch.

It was observed from *ex-vivo* permeation studies (Fig. 8) that flux of drugs from optimized cream(rutin and quercetin) was 0.1898 and 0.3481mg/hr/cm^2 respectively as compared to saturated solution of rutin and quercetin (0.1035 and 0.1264 mg/hr/cm^2) respectively.

CONCLUSION

Topical cream formulation of rutin and quercetin for venous diseases and varicose veins treatment which is patient compliant approach and economical. The formulation has been

optimized by DOE methodology and provide higher penetration to site of action. Thus, it can be better alternative to oral administration of rutin and quercetin. This study proves the ability of phytoconstituents with potential application to treat varicose veins with consequent health benefits.

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