FORMULATION, DEVELOPMENT AND EVALUATION OF *CISSUS QUADRANGULARIS* LINN. EXTRACT SUPPOSITORIES FOR THE TREATMENT OF HEMORRHOIDS.

Devashish Rane[1*], Dr. (Mrs.) Pallavi Chaudhari[2]

Abstract

The purpose of this study was to create suppositories with hydromethanolic extract of *Cissus quadrangularis* Linn. as a potential treatment for Hemorrhoids. Rapid absorption and high bioavailability are encouraged by the rectal route of drug delivery, which results in an early commencement of action. In-situ gelling suppositories were developed to address issues with first-pass metabolism, medication leakage, and patient discomfort. These suppositories were improved using the Box-Behnken design method with Design-Expert 13 software. The responses assessed included hardness, disintegration time, gelation temperature, and percentage of drug release. Combinations of PEG 600 (30%–50%), PEG 4000 (40%–60%), and Poloxamer 407 (5%–20%) were entered as factors. Tests for physical properties, weight fluctuation, and medication content were all passed by the improved formulation. These formulations displayed a melting point of around 36 ± 0.25 ℃, a pH between 6 and 7, a hardness of about 1.9 ± 0.06 kg/cm^2, a disintegration time of about 13 ± 0.47 minutes, a liquefaction time of about 10 ± 0.54 minutes, and an in-vitro drug release of 97.77 ± 0.47%. The extract also showed the abundance of bioflavonoids and other flavonoids in it as it was subjected for HPTLC analysis.

Keywords: *Cissus quadrangularis* Linn, Hemorrhoid, Macrogol, Poloxamer, Medicated Herbal Rectal Suppositories.

[1*]Research Scholar, Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Nigdi-Pradhikaran, Pune – 44, Maharashtra, India.
[2]Associate Professor, Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Nigdi-Pradhikaran, Pune – 44, Maharashtra, India. pallavichaudhari@dyppharmaakurdi.ac.in

***Corresponding Author:** Mr. Devashish Rane,
Research Scholar, Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-44.
E-mail ID: devarane99@gmail.com. Phone no.: +91 9834518946.

DOI: 10.48047/ecb/2023.12.si10.00420

Introduction

The vascular structures found in the anal canal are Hemorrhoids.[1] They serve as stools' controllable cushions in their usual state.[2] The phrases "Hemorrhoids" or "piles" are frequently used by lay people to describe this condition when they become large or irritating.[3] Hemorrhoids affect between 50% and 66% of people at some point in their lives.[4] Hemorrhoids most frequently affect people between the ages of 45 and 65, and the majority of them come from wealthy backgrounds.[5]

One of the most widely used medicinal plants in Thailand is a climber from the Vitaceae family called *Cissus quadrangularis* Linn. (Asthisanharak). Hemorrhoids and flatulence have both been treated with *C. quadrangularis*'s fresh aerial parts.[6] The usage of *C. quadrangularis* in Hemorrhoid patients was studied in a clinical experiment, and it was discovered that two 500 mg dry powder capsules taken twice daily were very efficient in treating hemorrhoidal pain and inflammation as well as shrinking the size of Hemorrhoids.[7] Phytochemical study of C. quadrangularis showed that there are compounds which possess pharmacological effects such as flavonoids, triterpenoids, phytosterol and Vitamin C.[8]

Suppositories are solid items that can vary in weight, size, and shape. They are intended to be put into a vaginal, urethral, or rectal orifice, where they melt, soften, or dissolve at body temperature to release the medication they contain.[9] Their formulation typically includes bases like cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, polyethylene glycols, etc..[10] A traditional suppository typically melts in the rectum at body temperature, which can eventually produce a number of issues, such as deprived patients experiencing discomfort in the anal region due to medication leakage. As it approaches the end of the rectum, a conventional suppository may also experience first-pass metabolism.[11] In order to create a rectal dosage form that forms a gel at body temperature, has proper gel strength, and does not leak out of the rectum after administration, the entire purpose of this study was to do that. Poloxamers are well recognised for their ability to show the phenomenon of reverse thermal gelation, which causes them to remain in solution at low temperatures (40 ℃) and gel at temperatures between 25 and 35 ℃.[12]

Materials

The drug *Cissus quadrangularis* Linn. hydromethanolic extract (CQ-HME) was generously gifted by Kisalaya Herbal Pvt. Ltd., Indore. Quercetin powder, PEG 400 and 600 were purchased from Loba Chemie Pvt. Ltd., Mumbai. PEG 4000 and 6000 were purchased from Research Lab Fine Chemical Industries, Mumbai. Poloxamer 407 was purchased from Analab Fine Chemicals, Mumbai. All other reagents and solvents used were of analytical grade.

Method

1. Preformulation studies

This study includes many preliminary tests in order to characterize the CQ-HME. These studies help to analyse and quantify the chemical constituents, extractible fractions, and other organic as well as inorganic matter.

1.1. Phytochemical screening

Various phytochemical tests were performed on the CQ-HME sample in order to examine the presence of various phytochemicals like flavonoids, tannins, saponins, alkaloids, glycosides, coumarins, etc.[13]

1.2. UV spectrophotometric study of CQ-HME

Stock solutions of CQ-HME (100 µg/ml) were prepared by dissolving an accurately weighed 10 mg quantity in 100 ml of distilled water. From the stock solution, 1 ml was diluted with distilled water to give solutions of 10 µg/ml concentration. The solutions were scanned in the range of 300 to 200 nm, and respective λ max values were reported in triplicate. The calibration curve of CQ-HME in distilled water was prepared in distilled water.

1.3. Physicochemical characteristics

The CQ-HME was exposed to the physicochemical criteria listed below. Total ash value, acid-insoluble, water-soluble, and ethanol-soluble extractives, and pH determination are all calculated. The physicochemical parameters' results are provided.[14-18]

1.4. DSC Analysis

DSC analysis was executed to assess the thermal constancy of the CQ-HME. Hitachi Model DSC 7020 (Nexta series) was employed to perform the DSC analysis. An alumina crucible was used to contain the 2.5 mg of CQ-HME in order to avoid temperature variations, as it was being measured by an exactitude thermocouple. The test was carried out in an N_2 atmosphere with a flow rate of

60 ml/min, with the samples being heated from 30 °C to 200 °C during the process at a 10 °C/min heating rate.[19]

1.5. Drug – Excipient Compatibility studies

The portions of CQ-HME were mixed with PEG 4000 and Poloxamer 407 respectively, and the formed mixtures were kept aside for 15 days. Changes in any of the physical or physicochemical characteristics of blends, if any, were observed visually and on the FT-IR spectrophotometry.

2. Preparation of trial batches

Suppositories were prepared using the moulding method.[20] Various PEGs were mixed manually for two to three minutes while melting at 60 °C in a water bath in a beaker. For all suppositories, the blends were cooled to a temperature of 50 – 55 °C and poured into a suppository mould of 2g capacity. They were first kept aside to set at 24 °C (room temperature) for 5 minutes and were later refrigerated at 10 °C for 10 minutes. The resulting suppositories were then trimmed and taken out of the mould. These suppositories were wrapped in aluminium foil and stored in individual plastic containers at room temperature (20 – 25 °C) for further analysis. The composition of various PEGs for the preparation of the 2 g suppositories for trial batches is shown in Table 1.

Table 1 : Composition of trial batches:-

Batch	Composition	Concentration
A	PEG 400 : PEG 4000	50:50
B	PEG 600 : PEG 4000	50:50
C	PEG 400 : PEG 6000	50:50
D	PEG 600 : PEG 6000	50:50

3. Evaluation of trial batches

3.3. Hardness

The hardness of the prepared suppositories was tested using a Monsanto hardness tester by holding the suppository diametrically in the fixed jaw of the tester.[21]

3.4. Melting point

For the melting point test, the suppository mass was placed in capillary tubes that were 10 cm long and filled to a height of 1 cm before being submerged in a beaker of water that was gradually heated on the heating mantle. It was noted at what temperature the bulk liquefies.[22]

3.5. Disintegration time

Six suppositories from each batch were taken, and their disintegration time was measured using a tablet disintegration test device (Electro lab, ED 2L). 500 ml of distilled water was used as a medium at 37 ± 0.5 °C. For suppositories prepared with water-soluble bases, the time required for complete disintegration of the suppositories was determined.[23]

3.6. Liquefaction time

One suppository from each batch was randomly chosen for this test. Thereafter, 60 mL of distilled water in a beaker was heated up to 37 ± 0.5 °C, and this temperature was maintained. Each suppository was dropped in the water, and the time taken for the suppository to completely dissolve was noted as the liquefaction time.[24]

4. Optimization of suppositories

This was carried out using Box-Behnken design on Stat Ease Design-Expert Software (Version 13.0.5.0). After comparing the results of the evaluation of various trial batches of suppositories prepared previously, independent factors and evaluated responses were selected as shown in Table 3.

Table 2 : Experimental variables used in Box-Behnken Design (BBD):

Sr. no.	Independent Variables	Actual values (coded)	
		Lowest (-1)	Highest (+1)
A)	**PEG 600**	30	50
B)	**PEG 4000**	40	60
C)	**Poloxamer 407**	5	20

Dependent variables:-

Y_1 - Hardness, Y_2 – Liquefaction time, Y_3 – Drug release, Y_4 – Gelation temperature.

5. Preparation of optimized batches of medicated suppositories

These suppositories were also prepared by the molding method. PEG 600, PEG 4000, and Poloxamer 407 were melted at 60 °C in a beaker using a water bath, and CQ-HME was dispersed under manual stirring for 2 to 3 min. After final mixing, the mixtures for all suppositories were cooled to a temperature of 50 to 55 °C and then poured into a suppository mould with a capacity of 2 g. They were first kept aside to set at 24 °C (room temperature) for 5 minutes and were later refrigerated at 10 °C for 10 minutes. The resulting suppositories were then trimmed and taken out of the mould. These suppositories were wrapped in aluminium foil and stored in individual plastic containers at room temperature (20 – 25 °C) for further analysis. The formulation chart for the preparation of a medicated suppository is shown in Table 4.

Table 3 : List of the formulations designed using Stat-Ease Design Expert Software (Version 13.0.5.0):

Formulations	**PEG 600 (%)**	**PEG 4000 (%)**	**Poloxamer 407 (%)**	**CQ-HME (%) (Total amount : 350 mg/ 2 gm of a suppository)**
F1	40	50	12.5	17.5
F2	30	40	12.5	17.5
F3	40	40	20	17.5
F4	50	50	5	17.5
F5	40	50	12.5	17.5
F6	30	60	12.5	17.5
F7	40	40	5	17.5
F8	40	60	5	17.5
F9	50	60	12.5	17.5
F10	50	50	20	17.5
F11	50	40	12.5	17.5
F12	40	50	12.5	17.5
F13	40	50	12.5	17.5
F14	40	50	12.5	17.5
F15	30	50	5	17.5
F16	40	60	20	17.5
F17	30	50	20	17.5

(% to be considered as parts)(**F1** is repeated as **F5, F12, F13,** and **F14**)

Figure 1 : Freshly prepared medicated CQ-HME suppositories.

6. Evaluation of optimized batches

Parameters like hardness, disintegration time, liquefaction time, and melting range were assessed using the same procedures that were followed during the assessment of the trial batches.

6.1. Drug Content

The average drug content of the medicated suppositories in each batch was estimated by randomly selecting three suppositories from each of those batches. Each suppository was dissolved using 5 ml of distilled water in a 100 ml volumetric flask, and the volume was made up to 100 ml with pH 6.8 phosphate buffer. The solution was then filtered and analysed after suitable dilutions for CQ-HME content at 200 nm using a UV-visible spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan).[25]

6.2.pH of the medicated suppositories

The pH of suppository formulations was determined by using a pH electrode connected to a digital pH metre. The pH metre was calibrated before each use with standard phosphate buffer (pH 7 and 4). One suppository from each batch was dissolved in 200 ml of distilled water for each measurement. The pH electrode was dipped in the previous suppository solution to determine the pH of that suppository.

6.3.Gelation temperature

Tube tilting method was used to measure the gelation temperature of medicated suppositories. For this, one suppository from each batch was melted in a test tube with the help of a water bath. Then the test tube was observed as the temperature of the molten mass was decreased in instalments of 1 °C and left to equilibrate for 5 min at each new setting. Gelation temperature was noted when the meniscus of the molten mass would no longer move upon tilting of test tube through 90°.[26]

6.4.In-vitro drug release

In vitro dissolution studies of CQ-HME medicated suppositories were carried out in a USP tablet dissolution test apparatus (Electro lab-TDT 08L) employing a rotating basket apparatus (USP Type I) at 50 rpm and using 500 ml of phosphate buffer (pH 6.8) at 37 ± 0.5 °C as dissolution medium. One suppository from each batch was used in each test. At pre-determined time intervals, 2.5 ml of samples were withdrawn by means of a pipette, which were then filtered through Whatman filter paper into 10 ml volumetric flasks individually. The temperature was kept around 37 ± 0.5 °C, and the volume that was withdrawn at regular intervals was replaced with an equal volume of fresh dissolving medium. The withdrawn samples were individually diluted up to 10 ml and then analysed for drug release by measuring the absorbance at 200 nm using a UV-visible spectrophotometer. In the end, the cumulative percent of CQ-HME released was calculated and plotted against time for each batch.[27]

7. HPTLC analysis

HPTLC analysis was carried out in order to quantify the presence of bioflavonoids in the CQ-HME, as the same set of compounds is proven to reduce the size and pain of hemorrhoids with the help of phlebotonic, vasculoprotective and antagonistic effect on the biochemical mediators of inflammation in hemorrhoids.[28] A CAMAG HPTLC system with a Linomat 5 applicator, a TLC scanner 3, a repro star 3, and a 12-bit CCD camera were utilized for picture documentation, all of which were managed by WinCATS-4 software. Ten milligrams of CQ-HME were diluted in ten milliliters of methanol and used as the test solution for HPTLC analysis. For the reference biomarker solution, 1 mg of quercetin was dissolved in 10 ml of methanol. The samples (10 µl) were spotted in 8 mm broad bands on a pre-coated silica gel glass plate 60 F-254 with a CAMAG microliter syringe. The sample-loaded plate was put in a TLC twin-trough developing chamber with the appropriate mobile phase (after being saturated with solvent vapor) and developed up to 83 mm in the appropriate mobile phase. The mobile phase comprised Toluene-Ethyl acetate-Glacial formic acid (5:4:0.2) (the solvent proportion was established by a preliminary TLC test). Linear ascending development in a 20 cm x 10 cm twin-trough glass chamber saturated with the mobile phase was followed by chromatoplate development in the same mobile phase to achieve good resolution of phytochemical contents. The best chamber saturation time for the mobile phase at room temperature was 30 minutes. To eliminate the solvents, the plate was dried using hot air. The plate was photographed at UV 382 nm and white light using a photo documentation chamber. Finally, the plate was scanned at 382 nm in the scanner stage. The plate was put in a photo-documentation chamber, and pictures were captured using white light, UV light at 304 and 382 nm, and infrared light at 304 and 382 nm. For densitometric scanning, the CAMAG TLC scanner III with CATS software (V 3.15, CAMAG) was employed.[29]

Figure no. 2: Preliminary TLC test carried out on drug extract and quercetin.

Results and discussion

1. Preformulation studies

1.1. Phytochemical screening

Table 4: Results of various phytochemical screening tests:

Test		**Result**
Test for Carbohydrates		
1	Molisch's test	+
2	Barfoed's test	+
3	Seliwanoff's Test	-
4	Test for starch (Iodine test)	+
Test for Reducing sugars		
1	Benedict's test	+
2	Fehling's test	+
Tests for Alkaloids		
1	Dragendroff's test	+
2	Hager's test	+
3	Mayer's test	+
4	Wagner's test	+
Tests for Flavonoids		
1	Alkaline reagent test	+
2	Ammonia test	+
3	Shinoda's test	+
4	Concentrated H_2SO_4 test	+
Tests for Steroids		
1	Salkowski's test	+
Tests for Phytosterols		
1	Libermann-Burchard's test	-
Tests for Phenols and Tannins		
1	Ferric chloride test	+
2	Bromine water test	+
3	Iodine test	-
4	Potassium dichromate test	+
Tests for Saponins		

1	Foam test	+
Tests for Glycosides		
1	Aqueous NaOH test	+
Tests for Cardiac glycosides		
1	Keller-Killani test	+
2	Baljet test	+
Tests for Anthroquinone glycosides		
1	Borntrager's test	-
Tests for Amino acids		
1	Ninhydrin test	-
Tests for Proteins		
1	Biuret test	-

(+ve:- Phytochemical test shows positive result, i.e., presence of phytoconstituent; -ve:- Phytochemical test shows negative result, i.e., absence of phytoconstituent)

This study helped to conclude that the CQ-HME had compiled phytoconstituents like flavonoids, alkaloids, saponins, cardiac glycosides, and phenolic compounds, which also implies that the quality of the CQ-HME complies with the set standard.[30]

1.2. UV spectrophotometric study of CQ-HME

The UV spectrum (λ max) of CQ-HME in distilled water indicated λmax at 200 nm. The standard calibration curve of CQ-HME in distilled water was found to be linear over the range of 2 -10 µg/ml as shown in Figure 2.

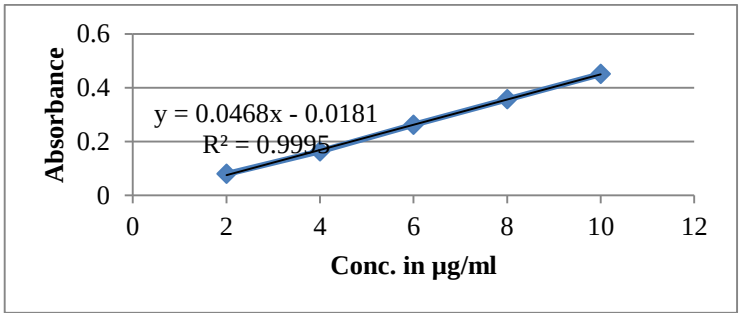

Figure 2: Calibration curve of CQ-HME in distilled water

1.3. Physicochemical characteristics

Table 5: List of various physicochemical characteristics along with their results:

Parameters	Result
Moisture content (Loss on drying)	5.82 ± 0.122%
Total ash value	5.5 ± 0.877%
Acid-insoluble ash value	2.5 ± 0.175%
Water soluble extractives	86.24 ± 0.317%
Alcohol soluble extractives	42.82 ± 0.682%
pH	4.37 ± 0.698

This study shows the results that comply with the standards.[31]

1.4. DSC analysis

Differential Scanning Calorimetry (DSC) is employed to measure the temperature and heat rate associated with transitions in materials as a function of time and temperature in a controlled atmosphere (N_2 atmosphere), which proves useful in the assessment of the thermal stability of the drug. The main parameters determined are: the glass transition temperature Tg, characterised by a change of the baseline in the DSC curve; specific heat (change of baseline); melting temperature, characterised by an endothermic peak in the DSC curve; crystallisation temperature (exothermic peak); oxidation (exothermic peak); degradation (peak endo or exothermic).[32] The DSC curve of CQ-HME is shown in Figure 3. The endothermic peak at 81.5 ℃ indicates a melting peak of CQ-HME, which implies that the addition of CQ-HME in a mixture or molten mass of excipients having a temperature below 81 ℃ is the only way to guarantee the thermal constancy of the same.

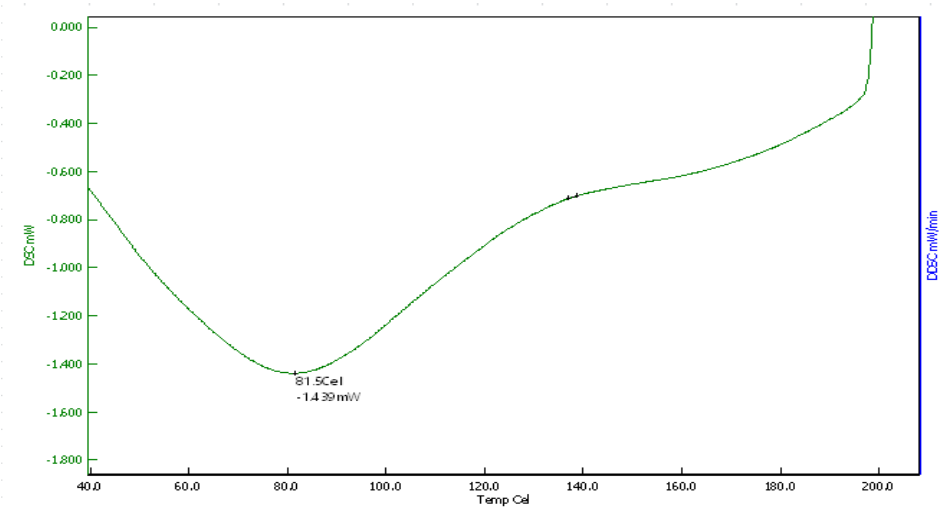

Figure 3: Thermographic curve of CQ-HME.

2. Evaluation of trial batches

Table 6 : Results of the evaluation of trial batches:

Parameters	**PEG 400 (50%) + PEG 4000 (50%) (Batch A)**	**PEG 600 (50%) + PEG 4000 (50%) (Batch B)**	**PEG 400 (50%) + PEG 6000 (50%) (Batch C)**	**PEG 600 (50%) + PEG 6000 (50%) (Batch D)**
Hardness	1.6 ± 0.98 kg/cm^2	**2.0 ± 0.09 kg/cm^2**	3.1 ± 0.25 kg/cm^2	4.5 ± 0.01 kg/cm^2
Melting point	33 ± 0.87 ℃	**36 ± 0.34 ℃**	38 ± 0.11 ^{0}C	41 ± 0.03 ℃
Disintegration time	12 ± 0.39 minutes	**13 ± 0.47 minutes**	15 ± 0.13 minutes	17 ± 0.06 minutes
Liquefaction time	14 ± 0.85 minutes	**14 ± 0.56 minutes**	6 ± 0.67 minutes	14 ± 0.82 minutes

After comparing these results with desired parameters for the ideal formulation (hardness in between 1.8 and 2.2 kg/cm^2, melting range in between 29 and 35 ℃, disintegration and liquefaction time below 30 minutes) [33], it becomes easy to conclude that the excipients from batch B (PEG 600 and PEG 4000) can be subjected to further optimisation as well as employment in the formulation.

3. Drug-Excipient Compatibility studies

The FT-IR spectrum (Figure 4) of CQ-HME showed major peaks at 3300 cm-1, 2859 cm-1, 1300 cm-1, 1060 cm-1, 841 cm-1 and 651 cm-1. The FTIR spectrum of the mixture of CQ-HME and excipients revealed that there was no interaction between the drug and excipients, as there was no major change in the position of the peaks of CQ-HME.

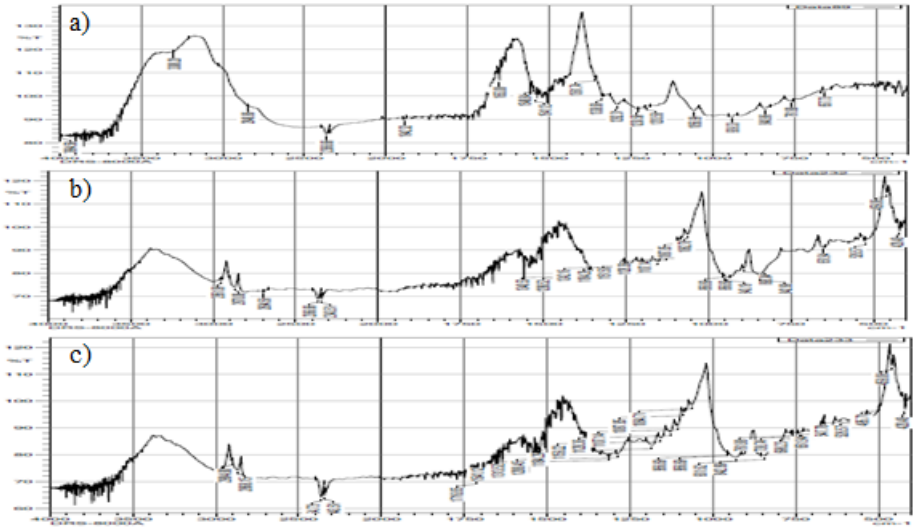

Figure 4: FTIR spectrum of a) CQ-HME alone & that of the b) physical mixture of CQ-HME with PEG 4000 and the c) physical mixture of CQ-HME with Poloxamer 407.

4. Evaluation of optimized batches

PEG 600 and PEG 4000 were decided to be accompanied by Poloxamer 407 in order to impart in-situ gel-forming ability to the resulting medicated formulation, as the latter had been widely used as the gelling agent in various formulations.

Table 7: Results of the evaluation of optimized batches:

Formulation	Hardness (kg/cm^2)	Liquefaction time (min)	Gelation temperature (^0C)	Melting point (^0C)	Drug content (mg/suppository)	% drug released
F1	5 ± 0.37	10 ± 0.15	32.7 ± 0.56	41 ± 0.21	348.00 ± 0.026	95.88 ± 0.46
F2	2.6 ± 0.34	12 ± 0.59	29.0 ± 0.36	37 ± 0.35	354.10 ± 0.049	94.29 ± 0.12
F3	2.5 ± 0.15	11 ± 0.13	30.8 ± 0.28	37 ± 0.32	346.79 ± 0.063	96.47 ± 0.45
F4	2.3 ± 0.59	12 ± 0.25	30.5 ± 0.24	36 ± 0.87	346.47 ± 0.095	96.19 ± 0.53
F5	5 ± 0.37	10 ± 0.15	32.7 ± 0.56	41 ± 0.21	348.00 ± 0.026	95.88 ± 0.46
F6	2.9 ± 0.36	11 ± 0.35	33.7 ± 0.79	43 ± 0.65	345.50 ± 0.060	95.36 ± 0.18
F7	2 ± 0.33	12 ± 0.54	31.6 ± 0.24	33 ± 0.23	335.89 ± 0.019	95.06 ± 0.43
F8	**1.9 ± 0.06**	**10 ± 0.54**	**33.3 ± 0.27**	**36 ± 0.25**	**349.44 ± 0.061**	**97.77 ± 0.47**
F9	2 ± 0.13	13 ± 0.14	30 ± 0.67	31 ± 0.83	348.51 ± 0.029	95.08 ± 0.24
F10	2.5 ± 0.12	13 ± 0.42	31.4 ± 0.35	34 ± 0.54	349.59 ± 0.058	96.37 ± 0.91
F11	2.8 ± 0.25	12 ± 0.59	29.9 ± 0.79	40 ± 0.13	347.15 ± 0.034	96.34 ± 0.26
F12	5 ± 0.37	10 ± 0.15	32.7 ± 0.56	41 ± 0.21	348.00 ± 0.026	95.88 ± 0.46
F13	5 ± 0.37	10 ± 0.15	32.7 ± 0.56	41 ± 0.21	348.00 ± 0.026	95.88 ± 0.46
F14	5 ± 0.37	10 ± 0.15	32.7 ± 0.56	41 ± 0.21	348.00 ± 0.026	95.88 ± 0.46
F15	1.9 ± 0.09	12 ± 0.36	33.2 ± 0.35	37 ± 0.63	345.52 ± 0.033	96.09 ± 0.34
F16	2.3 ± 0.11	12 ± 0.48	34.3 ± 0.54	36 ± 0.34	345.70 ± 0.018	94.67 ± 0.58
F17	2.2 ± 0.82	12 ± 0.52	31.9 ± 0.39	33 ± 0.72	347.13 ± 0.062	94.32 ± 0.42

All the formulations also showed a pH in the range of 6-7. The above-shown results helped to understand the effect of the addition of Poloxamer 407 on all the salient attributes, as the F8 batch having the least amount of Poloxamer 407 amongst all the batches showed the performance in terms of all the attributes in the desired manner (hardness in between 2 kg/cm^2, melting point in between 36 ℃, maximum % drug release within the given time, least liquefaction time, gelation temperature in between 31 and 36 ℃, pH in the range of 6-7). Hence, F8 had the privilege of being chosen as the best fit for an optimised batch.

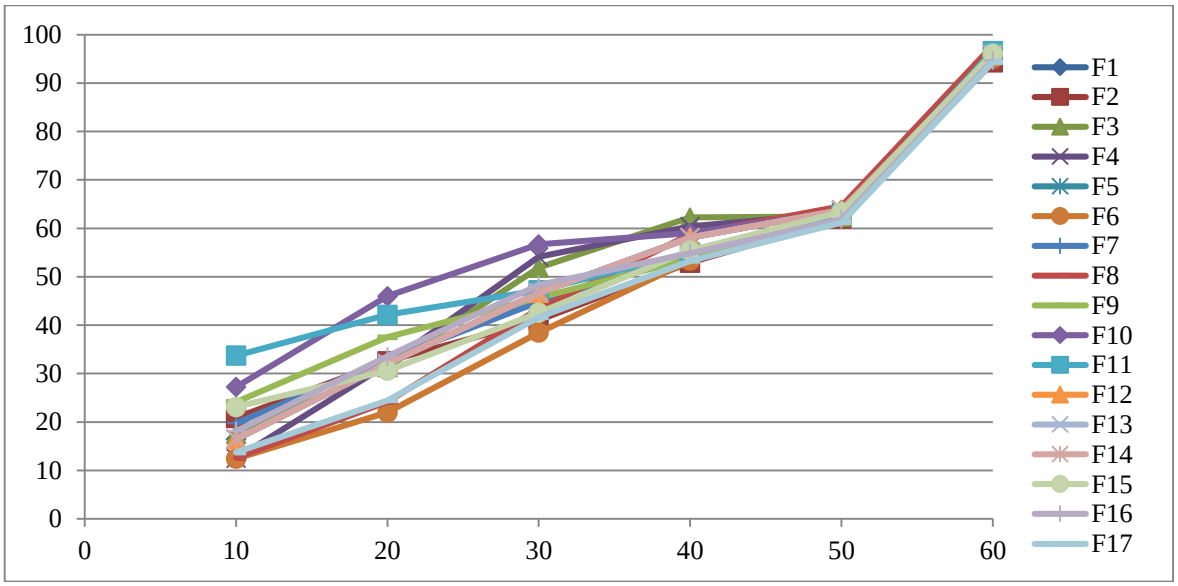

Figure 5: Dissolution plots of cumulative drug release of CQ-HME suppository of above formulation.

5.Experimental design

The independent variables to prepare suppository formulations from mixture design and evaluated responses have already been mentioned in Table 3.

5.1. Effect of independent variables on the hardness (Y_1)

The quadratic model was found to be significant after applying the Box Behnken Design, with a model F value of 75.67 and a p value < 0.001. The following quadratic equation describes the effect of concentrations of PEG 600, PEG 4000, and Poloxamer 407 on the hardness of suppositories.

Hardness (kg/cm^2) = 5.00 – 0.1000B + 0.1750C - 0.2750AB - 0.0250AC - 0.0250BC - 1.19A^2 - 1.24B^2 -1.59C^2.

A positive value on the factors of the equation means hardness and that the factors are directly proportional to each other, while a negative value in the equation implies that they are inversely proportional to each other. The hardness decreases with the reduction in the concentration of Poloxamer 407 (factor C). At the medium level of PEG 600, the high level of PEG 4000, and the low level of Poloxamer 407, an optimised formulation F8 showed hardness at 1.9 kg/cm^2. An interaction between those three factors and hardness as an evaluated response is shown as a 3D response surface plot in Figure 6 a) and a contour plot in Figure 6 b).

Table 8: Summary of ANOVA for hardness parameters.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	26.27	9	2.92	75.67	< 0.0001 (S)
Residual	0.2700	7	0.0386	-	-
Total	26.54	16	1.659	-	-

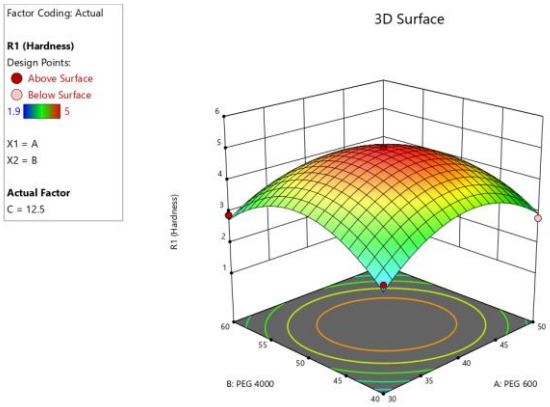

Figure 6: a) 3-D Response surface plot showing the effect of PEG 600 and PEG 4000 on the hardness of suppositories.

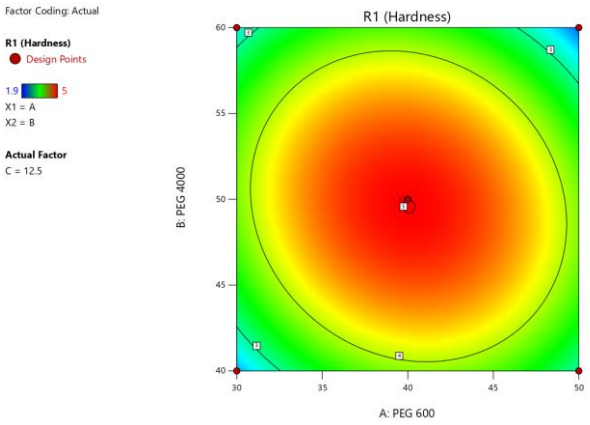

Figure 6: b) the Contour plot showing the effect of PEG 600 and PEG 4000 on the hardness of suppositories.

5.2. Effect of independent variables on liquefaction time (Y_2)

The quadratic model was found to be significant after applying the Box Behnken Design, with a model F value of 59.98 and a p value < 0.001. The following quadratic equation describes the effect of concentrations of PEG 600, PEG 4000, and Poloxamer 407 on the liquefaction time of suppositories.

Liquefaction time (min) = 10.00 + 0.3750A - 0.1250B + 0.2500C + 0.5000AB + 0.2500AC + 0.7500BC + 1.50A^2 + 0.5000B^2 + 0.7500C^2.

The liquefaction time also decreases with the reduction in the concentration of Poloxamer 407 (factor C). At the medium level of PEG 600, the high level of PEG 4000, and the low level of Poloxamer 407, an optimised formulation F8 showed 10 minutes of liquefaction time. An interaction between those three factors and liquefaction time as an evaluated response is shown as a 3D response surface plot in Figure 7 a) and a contour plot in Figure 7 b).

Table 9 : Summary of ANOVA for liquefaction time parameters.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	19.28	9	2.14	59.98	< 0.0001 (S)
Residual	0.2500	7	0.0357	-	-
Total	19.53	16	1.221	-	-

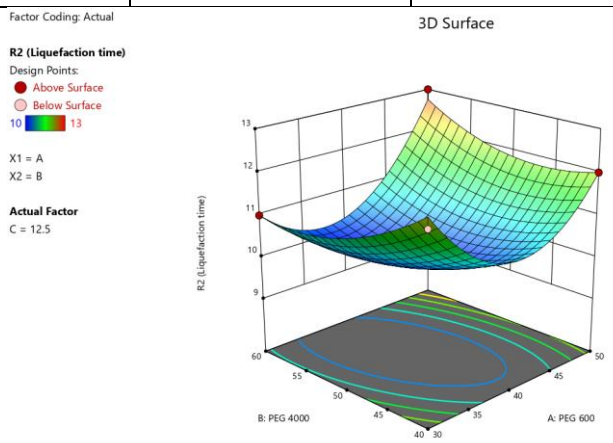

Figure 7: a) 3-D Response surface plot showing the effect of PEG 600 and PEG 4000 on the liquefaction time of suppositories.

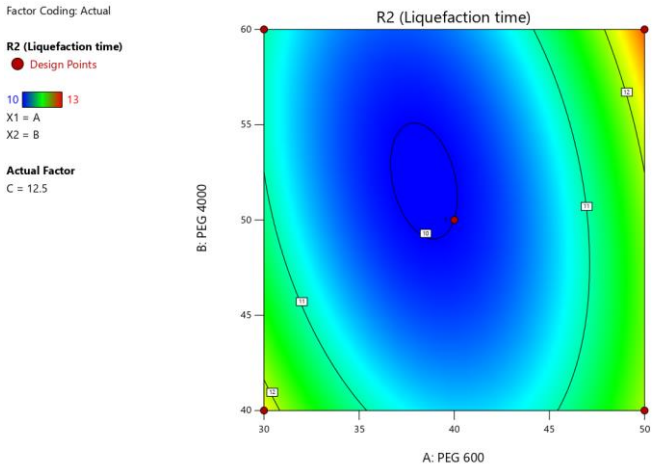

Figure 7: b) the Contour plot showing the effect of PEG 600 and PEG 4000 on the liquefaction time of suppositories.

5.3. Effect of independent variables on percentage drug release (Y_3)

The quadratic model was found to be significant after applying the Box Behnken Design, with a model F value of 53.75 and a p value < 0.0001. The following quadratic equation describes the effect of concentrations of PEG 600, PEG 4000, and Poloxamer 407 on the percentage drug release of suppositories.

Drug release per hour (%) = 95.88 + 0.4903A + 0.0914B - 0.4087C - 0.5816AB + 0.4896AC - 1.13BC - 0.4324A^2 - 0.1838B^2 + 0.2945C^2.

As an aspect of significance, the percentage of drug release was found to be inversely proportional to the concentration of Poloxamer 407 (factor C). At the medium level of PEG 600, the high level of PEG 4000, and the low level of

Poloxamer 407, an optimised formulation F8 showed around 97.77% drug release. An interaction between those three factors and percentage drug release as an evaluated response is shown as a 3D response surface plot in Figure 8 a) and a contour plot in Figure 8 b).

Table 10 : Summary of ANOVA for drug release parameters.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	11.97	9	1.33	53.75	< 0.0001 (S)
Residual	0.1733	7	0.0248	-	-
Total	12.15	16	0.759	-	-

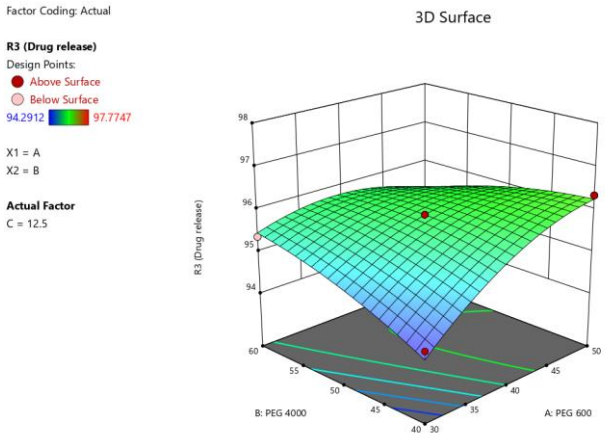

Figure 8: a) Response surface plot showing the effect of PEG 600 and PEG 4000 on the % drug release of suppositories.

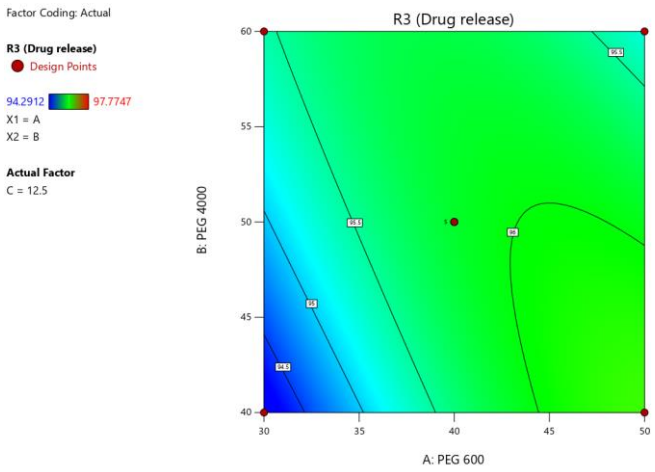

Figure 8: b) the Contour plot showing the effect of PEG 600 and PEG 4000 on the % drug release of suppositories.

5.4. Effect of independent variables on gelation temperature (Y_4)

The quadratic model was found to be significant after applying the Box Behnken Design, with a model F value of 322.62 and a p value < 0.0001. The following quadratic equation describes the effect of the concentrations of PEG 600, PEG 4000, and Poloxamer 407 on the gelation temperature of the suppositories.

Gelation temperature (^{0}C) = 32.7 - 0.75A + 1.25B - 0.0250C - 1.15AB + 0.55AC + 0.45BC - 1.4A^2 - 0.65B^2 + 0.45C^2.

At the medium level of PEG 600, the high level of PEG 4000, and the low level of Poloxamer 407, an optimised formulation F8 showed a gelation temperature of around 33.3 °C. An interaction between those three factors and gelation temperature as an evaluated response is shown as a 3D response surface plot in Figure 9 a) and a contour plot in Figure 9 b).

Table 11: Summary of ANOVA for gelation temperature parameters.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	35.26	9	3.92	322.62	< 0.0001 (S)
Residual	0.0850	7	0.0121	-	-
Total	35.34	16	2.209	-	-

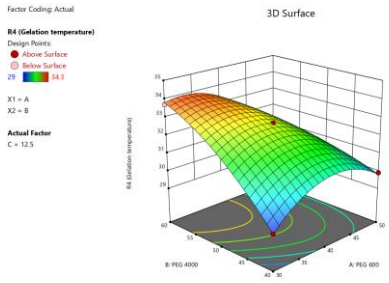

Figure 9: a) 3-D Response surface plot showing combined effect of PEG 600 and PEG 4000 on the gelation temperature.

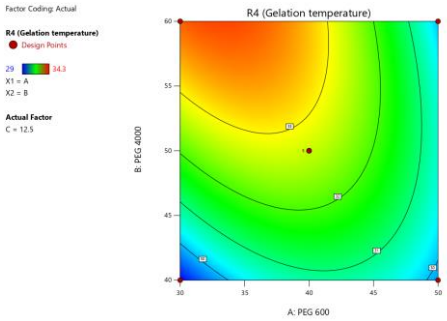

Figure 9: b) the Contour plot showing combined effect of PEG 600 and PEG 4000 on the gelation temperature.

Table 12: Regression analysis of responses.

Response (denotation)	Adjusted R^2	Predicted R^2	Adequate precision
Hardness (Y_1)	0.9767	0.8372	20.41
Liquefaction time (Y_2)	0.9707	0.7952	21.56
% drug release (Y_3)	0.9674	0.7718	29.15
Gelation temperature (Y_4)	0.9945	0.9615	61.23

6. HPTLC analysis

High Performance Thin Layer Chromatography (HPTLC) is the simplest and quickest separation technology available today, providing more precision and accuracy while providing extraordinary flexibility for diverse processes. Table 2 displays the findings, including the number of peaks, highest Rf value, and total% area. CQ-HME exhibited a single peak in the 200-800 nm spectral region.

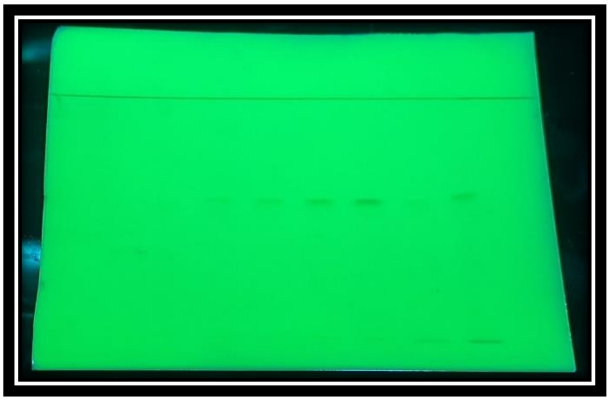

Figure no. 10: UV screening of HPTLC plate showing extract and quercetin bands.

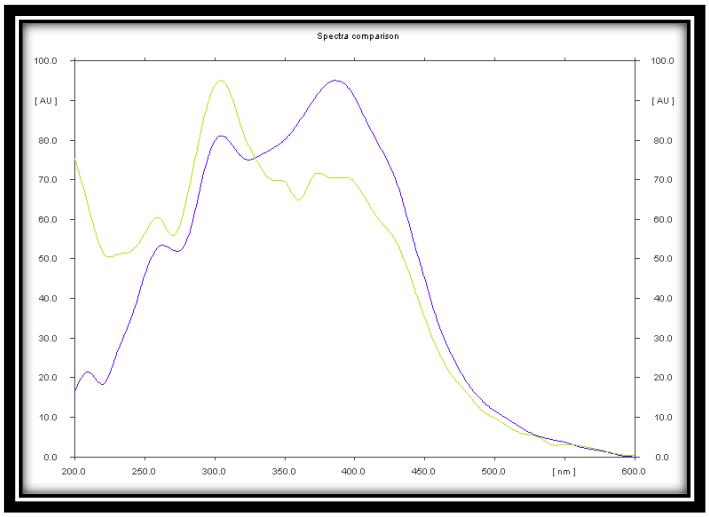

Figure no. 11: Overlain spectra of 0.4 µl quercetin and 5 µl drug extract.

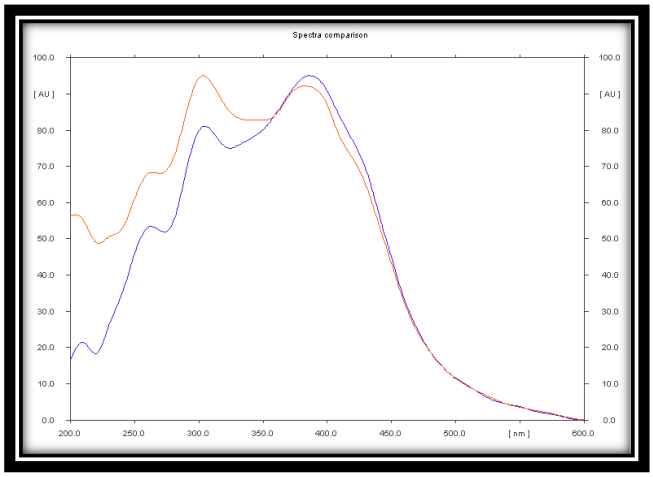

Figure no. 12: Overlain spectra of 0.4 µl quercetin and 10 µl drug extract.

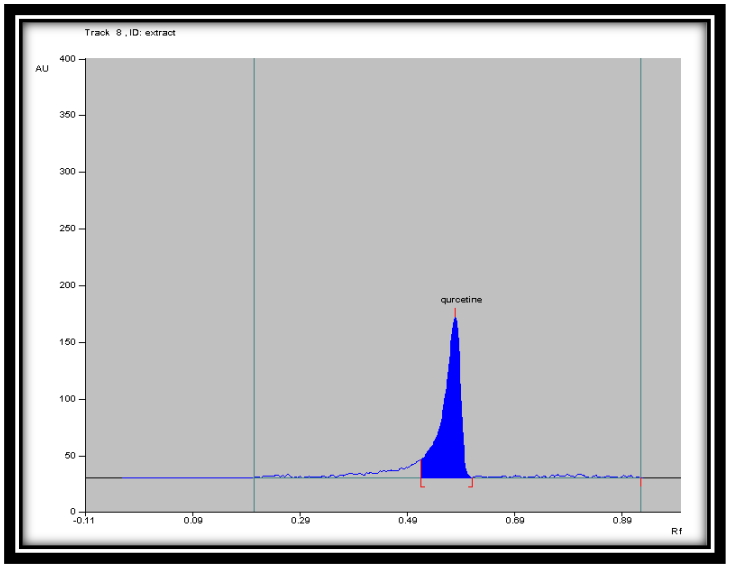

Figure no. 13: Final HPTLC chromatograph.

Table 13: HPTLC spectral analysis of CQ-HME.

Peak	**Max R_f**	**Area (%)**
1	0.58	100.0

Based on the above findings, it can be stated that the *Cissus quadrangularis* Linn. medicinal extract includes a significant quantity of bioflavonoids such as quercetin, diosmin, hesperidin, and others, which can be beneficial in reducing the discomfort and inflammation associated with hemorrhoids, with their analgesic, venotonic and anti-inflammatory actions.

Conclusion

The medicated CQ-HME suppositories belonging to the formulation named F8, made up of equal but high proportions of both PEG 600 and PEG 4000 and the lowest proportion of Poloxamer 407, were found to be the best fit in terms of stability as well as desired attributes. Suppositories from the same formulation passed the criteria of the weight variation test and the drug content assay as well. Those suppositories also showed a hardness of 1.9 kg/cm^2, liquefaction time of 10 minutes, a disintegration time of 17 minutes, a melting range of 30 - 35 ℃ and a drug release rate of approximately around 98%. The formulation also proved its efficient in-situ gelling ability, as it also showed gelation at 33.3 ℃. This study also proved that selected factors like concentrations of PEG 600, PEG 4000, and Poloxamer 407 had an impact on the above evaluated responses. The CQ-HME also showed that its attributes comply with the given standards. The HPTLC analysis helped to conclude that the bioflavonoids, compounds responsible to reduce the size and pain of the piles, are present in the CQ-HME extract. The same HPTLC analysis also convinced that the efficacy of the formulation can be proven without conducting animal trials. Thus it can be concluded that formulations developed using CQ-HME can perform well in terms of pharmacokinetics as an herbal rectal treatment for Hemorrhoids, free from side effects, in the patients.

Acknowledgments

Authors would like to express their sincere gratitude to Kisalaya Herbal Pvt. Ltd., Indore. For their generous gift of The drug *Cissus quadrangularis* Linn. hydromethanolic extract (CQ-HME) and Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for performing DSC and HPTLC analysis on the Cissus quadrangularis Linn. hydromethanolic extract (CQ-HME) sample on behalf of the authors.

Conflicts of interests

Authors declare no conflict of interests.

ORCID IDs

Dr. (Mrs.) Pallavi Chaudhari https://orcid.org/0000-0002-8426-6601

References

1. Schubert MC, Sridhar S, Schade RR, Wexner SD. What every gastroenterologist needs to know about common anorectal disorders. World journal of gastroenterology: WJG. 2009 Jul 7; 15(26):3201.
2. Fleshman JW, Wolff BG, Beck DE, Wexner SD, Pemberton JH, editors. The ASCRS textbook of colon and rectal surgery. Springer Science & Business Media; 2007. 175.
3. Johanson JF, Sonnenberg A. The prevalence of hemorrhoids and chronic constipation: an epidemiologic study. Gastroenterology. 1990 Feb 1; 98(2):380-6.
4. Lorenzo-Rivero S. Article Commentary: Hemorrhoids: Diagnosis and Current Management. The American Surgeon. 2009 Aug; 75(8):635-42.
5. Sun Z, Migaly J. Review of hemorrhoid disease: presentation and management. Clinics in colon and rectal surgery. 2016 Mar; 29(01):022-9.
6. Chopra SS, Patel MR, Awadhiya RP. Studies of *Cissus quadrangularis* in experimental fracture repair: a histopathological study. The Indian journal of medical research. 1976 Sep 1; 64(9):1365-8.
7. Segsunviriya C, Choomprabutra S. A clinical study of Cissus quadrangularis Linn. in hemorrhoid patients. In: Seminar of Research and Development of Medicinal Plant. Division of Medical Research, Department of Medical Sciences, Ministry of Public Health, Thailand; 1989. p. 54-55.
8. Bhutani KK, Kapoor R, Atal CK. Two unsymmetric tetracyclic triterpenoids from *Cissus quadrangularis*. Phytochemistry. 1984 Jan 1;23(2):407-10.
9. Lowry M. Rectal drug administration in adults: how, when, why. Nursing Times. 2016 Feb 1; 112(8):12-4.
10. Remington JP. Remington: the science and practice of pharmacy. Lippincott Williams & Wilkins; 2006. 884-85.
11. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK. Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. International journal of pharmaceutics. 1998 Apr 20; 165(1):33-44.
12. Jaafar IS, Radhi AA. Formulation and in vitro evaluation of in situ gelling liquid suppository

of promethazine HCL. WJPR. 2015 Sep 14; 4:273-86.
13. Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008 Sep 7. 25.1-9.
14. Pandey P, Mehta R, Upadhyay R. Physico-chemical and preliminary phytochemical screening of Psoralea corylifolia. Archives of Applied Science Research. 2013;5(2):261-5.
15. Malika NA, Mohamed FA, Chakib EA. Microbiological and physicochemical properties of Moroccan honey. International Journal of Agriculture and Biology. 2005 Jul;7(5):773-6.
16. Baravalia Y, Nagani K, Chanda S. Evaluation of pharmacognostic and physicochemical parameters of Woodfordia fruticosa Kurz. Flowers. Pharmacognosy Journal. 2011 Jan 1;2(18):13-8.
17. Taylor AM, Freitag C, Cadot E, Morrell JJ. Potential of near infrared spectroscopy to assess hot-water-soluble extractive content and decay resistance of a tropical hardwood. Holz als Roh-und Werkstoff. 2008 Apr 1;66(2):107-11.
18. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of Averrhoa carambola L. fruit. J Herb Med Toxicol. 2008;2(2):51-4.
19. Manimekalai G, Kavitha S, Divya D, Indran S, Binoj JS. Characterization of enzyme treated cellulosic stem fiber from *Cissus quadrangularis* plant: an exploratory investigation. Current Research in Green and Sustainable Chemistry. 2021 Jan 1; 4:100162.
20. Christ AP, Biscaino PT, Lourenço RL, de Souza AB, Zimmermann ES, Adams AI. Development of doxycycline hyclate suppositories and pharmacokinetic study in rabbits. European Journal of Pharmaceutical Sciences. 2020 Jan 15; 142:105141.
21. Baviskar P, Jaiswal S, Sadique S, Landged A. Formulation and evaluation of lornoxicam suppositories. The Pharma Innovation. 2013 Sep 1; 2(7, Part A):20.
22. Kauss T, Gaubert A, Boyer C, Ba BB, Manse M, Massip S, Léger JM, Fawaz F, Lembege M, Boiron JM, Lafarge X. Pharmaceutical development and optimization of azithromycin suppository for paediatric use. International journal of pharmaceutics. 2013 Jan 30; 441(1-2):218-26.
23. Sudke SG. Design of curcumin suppositories for effective treatment of vaginal candidiasis using cow ghee as a suppository base. Asian Journal of Pharmaceutics (AJP). 2017;11(04).
24. Isimi CY, John-Africa LB, Ekere KE, Olayemi OJ, Aremu OI, Emeje MO. Formulation, evaluation and anti-hemorroidal activity of suppositories containing moringa oleifera lam. seed oil. ACTA Pharmaceutica Sciencia. 2021; 59(1).
25. De Muynck C, Lefebvre RA, Remon JP. Study of the bioavailability of four indomethacin suppository formulations in healthy volunteers. International journal of pharmaceutics. 1994 Apr 8;104(1):87-91.
26. Lenaerts V, Triqueneaux C, Quartern M, Rieg-Falson F, Couvreur P. Temperature-dependent rheological behavior of Pluronic F-127 aqueous solutions. International journal of pharmaceutics. 1987 Sep 1; 39(1-2):121-7.
27. Ghazwani M, Hani U, Osmani RA, Rahamathulla M, Begum MY, Wahab S, Gowda DV, Ravikumar AA, Kumar HY, Ather H, Moin A. An efficient herbal approach for treating fungal infection in cervical cancer patients by developing and optimizing a vaginal suppository. International Journal of Polymer Science. 2021 Nov 5; 2021:1-1.
28. Cospite M. Double-blind, placebo-controlled evaluation of clinical activity and safety of Daflon 500 mg in the treatment of acute hemorrhoids. Angiology. 1994 Jun 2;45.
29. Chanda S, Baravalia Y, Nagani K. Spectral analysis of methanol extract of *Cissus quadrangularis* L. stem and its fractions. Journal of Pharmacognosy and Phytochemistry. 2013Sept3;2(4):149-57.
30. Srivastava AK, Srivastava P, Behera BR, Shrivastava AK. Pharmacognostical & phytochemical investigation of *Cissus quadrangularis* Linn. Stem. Int J Pharm Re s Dev. 2011; 3:207-15.
31. Indian Pharmacopoeia, Vol. II, 3rd edn. Ministry of health and family welfare, Govt. of India, Controller of publications, New Delhi 1985, p. A-53.
32. Xie F, Liu WC, Liu P, Wang J, Halley PJ, Yu L. Starch thermal transitions comparatively studied by DSC and MTDSC. Starch-Stärke. 2010 Jul; 62(7):350-7.
33. Abd Ellah NH, Shaltout AS, Abd El Aziz SM, Abbas AM, Abd El Moneem HG, Youness EM, Arief AF, Ali MF, Abd El-hamid BN. Vaginal suppositories of cumin seeds essential oil for treatment of vaginal candidiasis: Formulation, in vitro, in vivo, and clinical evaluation. European Journal of Pharmaceutical Sciences. 2021 Feb 1; 157:105602.