RESEARCH ARTICLE

Fabrication, Optimization and Evaluation of Escin Enriched Emulgel System for Treatment of Varicose Veins

Chougule PN[1,2*], Koumaravelou K[1], Chougule NB[3]

[1]*Department of Centre for Research and Development, PRIST University, Vallam, Thanjavur, Tamil Nadu, India.*
[2]*Ashokrao Mane College of Pharmacy, Kolhapur, Maharashtra, India*
[3]*Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur, Maharashtra, India*

Received: 04th January, 2023; Revised: 07th June, 2023; Accepted: 12th August, 2023; Available Online: 25th September, 2023

ABSTRACT

Varicose veins, a widespread chronic venous ailment in lower limbs, impact many individuals, causing considerable physical, financial, and social burdens. While surgery is the most effective treatment, its cost hinders personalized care. In the quest for venous-specific therapies, herbal remedies, particularly horse chestnut extract containing escin, are being explored. Escin, a key component, offers anti-inflammatory, anti-edematous, and antioxidant effects. The saponin escin has two forms, α and β, with distinct actions via oral and topical routes. A gel system with permeability enhancers was developed to enhance topical escin's effectiveness following meticulous pre-formulation and optimization studies. The physiochemical characteristics of emulsion and emulgel system, assay of escin, *in-vitro* permeation, emulgel studies such as pH, viscosity, spreadability and stability studies are all included in the evaluation part. The 2% escin in the light beige-colored emulgel system was significantly improved permeability when soy lecithin was added. The investigation on permeation enhancement shows that there is 1.5 times more activity than there would be with standard preparation. Escin's concentration was measured using a UV spectrophotometer and was found to be between 98 and 102%. Studies such as pH, viscosity and spreadability are within the predefined limits. Overall, the developed escin-based emulgel system shows promising results for the management of varicose veins.

Keywords: Varicose vein, Horse chest nut, Escin, Emulgel, Topical gel.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.3.10

How to cite this article: P N Chougule, K Koumaravelou, NB Chougule. Fabrication, Optimization and Evaluation of Escin Enriched Emulgel System for Treatment of Varicose Veins. International Journal of Drug Delivery Technology. 2023;13(3):833-841.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The word 'varicose' has its roots in the Latin term 'Varix,' which signifies 'twisted.' The World Health Organization (WHO) provides a definition for varicose veins as veins exhibiting a tortuous saccular development. The concept of "Varicosity" is commonly employed to characterize vessels that have become elongated, tortuous, pouched, thickened, and brittle, resulting in a permanent loss of their valve efficiency. Comparable changes can also manifest in veins, leading to chronic venous insufficiency of the lower limb-a condition encompassing diverse indications and symptoms arising due to venous hypertension. Varicose veins represent a venous disorder that results in the disruption of normal blood flow, causing turbulence in circulation. This condition leads to the abnormal enlargement of veins due to edema. It is influenced by genetic factors, as well as the presence of weakened vascular walls, incompetent valves, and elevated intravenous pressure. Indications of varicose veins encompass sensations of heaviness, discomfort, itching, and burning. These symptoms tend to worsen during prolonged periods of standing. Age, pregnancy, hereditary, obesity, employment, which include prolonged hours of standing, types of physical activity, and diet are the risk factors. Notable approaches for treatment include external laser procedures, injection sclerotherapy, endovenous interventions, and surgical methods. The selection of themost suitable treatment is influenced by elements like the patient's symptoms, personal preferences, financial considerations, the threats of potential complications originating from treatment, and the accessibility of medical resources. The intention of medicinal plants to cure the alignment has been a universal practice earth-wide. Due to its cultural and historical reasons, its acceptance is still maintained. The use of medicinal plants remains pivotal in healthcare systems, particularly within developing nations, where herbal remedies have a well-established tradition of extensive utilization. Especially in Asian countries like India, Japan, China, Shri-Lanka, Pakistan, and Thailand, the traditional medicine practice is ubiquitous.

**Author for Correspondence: pnchougule1008@gmail.com*

Many traditional and natural treatment options, such as plants containing potential phytoconstituents which are scientifically proven and validated with some sort of scientific pre-clinical data. With respect to usage of herbal extracts for developing formulations lacks proper clinical efficacy due to the presence of other ingredients or sometimes the less quantity within the extract. Scientifically reported phytoconstituents for the management of varicose veins encompass *Centella asiatica* extract, horse chestnut seed extract, butcher's broom extract, apple cider vinegar, amla extract, garlic extract, and grape seed extract.

Out of the well-researched plant extracts, the notable horse chestnut seed extract is a rich source of important phytoconstituents with potential venotonic properties. Horse chestnut seed has botanical name is, *Aesculus hippocastanum*. The primary and main active constituent of horse chestnut seed is escin. Figure 1 depicts the structure of escin.

The α and β of these two triterpene saponins are present in extract, differentiated by their aqueous solubility and physical constant. The 'α' form of escin is orally active, and the 'β' form of escin is topically active. In addition, some other compounds are also present: proanthocyanidines, bioflavonoids, and coumarins. The β escin is very effective in the saponins phyto-bunch and it is used in pharmaceuticals which is useful in chronic venous insufficiency. Escin's ability to hinder hyaluronidase activity doesn't entirely elucidate the robust venotonic impacts of horse chestnut seed extracts on microvasculature. These extracts might also impede other enzymes such as collagenase, elastase, and β glucuronidase, which contribute to upholding the integrity of the extravascular matrix.

Topical drug delivery approach is among the attractive routes for local as well as systemic treatment. Topical emulgel product has many advantages than conventional dosage forms. Topical formulations showed its action directly to the targeted site.

Therefore, present study attempts to fabricate optimize and evaluate escin, the principal active constituent of horse chest nut seed extract into the advanced emulgel system in a patient-friendly and desired performance attribute.

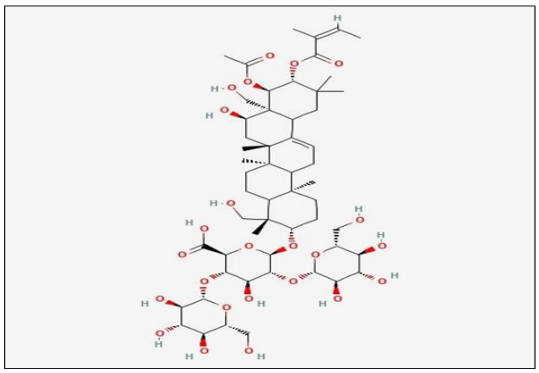

Figure 1: Chemical structure of escin

MATERIALS AND METHODS

Materials

Escin was obtained from Simson Pharma Ltd Mumbai. Paraffin oil–Arihant Sidha Lab Sangli. Steric acid, white beeswax, cetyl alcohol, and ceto stearyl alcohol –Research Lab Fine Chem Industry Mumbai. Soya lecithin and propylene glycol – Loba chemicals. TEA 88% and phenoxyethanol–Galaxy Surfactants Mumbai. Carbopol 980- Corel Pharma Chem Ahmadabad. The rest of the chemicals and reagents used are of high purity.

Methodology

Preformulation studies

Preformulation studies mark the initial stage in the rational design of a drug substance's dosage form. These assessments strive to identify the physicochemical properties and excipients that might influence the formulation and the manufacturing process. Preformulation studies such as determination of melting point by capillary method, FTIR spectroscopy, solubility in different solvents and visual inspection studies were performed.

Development of UV spectroscopy method of escin for routine analysis

Standard solution and sample solution at a concentration of 1-mg/mL was prepared with purified water in 100 mL volumetric flask using escin. From this resultant solution, a concentration series of further dilutions was created. Finally, working standards with a series were prepared of strength 30.00, 40.00, 50.00, 60.00, 70.00, 80.00, 90.00, 100.00 µg/mL with the addition of distilled water in 10 mL volumetric flask. All prepared stock solutions and sample solutions were scanned between UV ranges. Finally, the obtained λ_{max} was 305 nm was chosen and utilized for further analysis.

Formulation and optimization of escin-enriched emulgel system

The homogenous emulsion system of escin was formulated by a high-speed overhead homogenizermixer utilizing the different ratio of oil phase components with the selected material. A total 4 trialbatches were executed and shown in Table 1, Figure 2 (VL-01 to VL-04).

Preparation of aqueous phase: An accurately weighed quantity of phenoxyethanol was dispersed with a small amount of propylene glycol followed by the addition of soya lecithin, to which the quantity of purified water was dropped. To this resulting solution, the weighed quantity of escin was dispersed and heated to attain a temperature of 70°C. Formulation of oil phase 'B': Accurately weighed amount of liquid paraffin oil, cetyl alcohol, cetostearyl alcohol, white bees wax and stearic acid in a stainless still vessel heated to melt all components to liquid state and mixed together by achieve a temperature of 70°C.

Combining solution 'A' with dispersion 'B', both phases were thoroughly mixed using a high-speed overhead homogenizer while maintaining the appropriate rpm to achieve a fully homogeneous emulsion system.

Table 1: Compositions of escin emulgel system

Sr. No	*Phase*	*Ingredients*	*VL-01*	*VL-02*	*VL-03*	*VL-04*
1	Oil Phase Components	Stearic acid	3.0	3.0	2.0	2.0
2		White beeswax	3.0	3.0	2.0	2.0
3		Ceto stearyl alcohol	5.0	5.0	4.0	4.0
4		Cetyl alcohol	7.0	7.0	5.0	5.0
5		Liquid paraffin	10.0	10.0	10.0	10.0
6		Propylene glycol	10.0	10.0	10.0	10.0
7		TEA 88%	2.0	2.0	2.0	2.0
8		Phenoxyethanol	0.1	0.1	0.1	0.1
9	Water Phase Components	Soya lecithin	0.2	0.3	0.5	0.5
10		Carbomer 980	0.5	0.8	0.7	0.7
11		Escin powder	2.0	2.0	2.0	2.0
12		Saline water	Q.S	Q.S	Q.S	Q.S

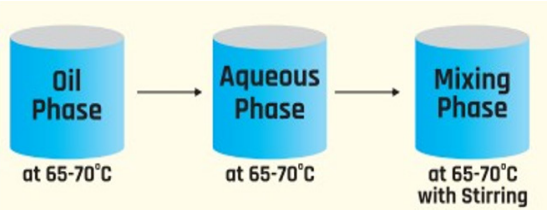

Figure 2: High speeds emulsion formation process

Preparation of emulgel system with the support of carbomer polymers

In the quest for an optimal topical formulation for skin delivery, two critical aspects are the ease of application onto the skin and the ability to possess appropriate rheological attributes. Integrating aqueous escin emulsion dispersions into gels to attain the semisolid texture akin to professional applications. Carbomer 980 (0.5 to 0.8% w/w) was previously soaked in water for 8 hours. Shown in Figure 3, This required a quantity of triethanolamine incorporated for cross-linking of carbomer to form gel system. This gel system previously developed escin homogenous emulsion dispersions was incorporated under continuous stirring to have smooth homogenous emulgel system to attain the desired semisolid consistency.

Characterization Studies of Escin-Based Emulgel System

Evaluation of physiochemical properties of emulsion and emulgel system

Few physiochemical properties such as description, odor and texture were evaluated. Olympus Microscope and

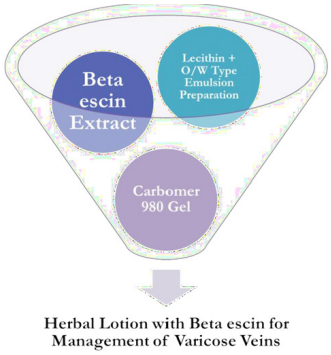

Figure 3: Composition system of Escin Emulgel

LABLINE optical Microscope to notice homogeneity of emulgel.Any alteration in color, transparency, or occurrence of phase separation was noted under standard conditions with maintaining ambient (37°C) for the optimized final formulation.

Photomicroscopy

The optimized emulgel product was checked with the help of photomicroscopy, checking homogeneity and good consistency.

Identifying the critical quality attributes (cqas) through risk analysis

Critical quality attributes for developing efficacious topical gel system are one of the important studies in which we identify crucial ingredients and their respective concentrations which have direct impact on formulation aspects and stability. This study is called as identification of product parameters. We also have studied the process parameters where temperature, mixing speed and cooling temperature directly impact physiochemical properties. Based on product and process parameter identification, there are greater chances to have a homogeneous, consistent and efficacious formulation of escin topical gel.

pH

The pH of escin-based formulated gels was determined by utilizing a digital pH meter by diluting 10% of formulation with sufficient purified water.

Determination of viscosity

The viscoelastic characteristics of the topical emulgel were estimated using the Brookfield viscometer RV model with 64 no spindle at rpm 10 and torque 74.6 using a small sample adaptor. Data analysis was done with excel sheet. The rheological characteristics of the optimized gels containing enriched escin were examined through continuous shear testing utilizing Brookfield equipment. The gel was equilibrated at 25 ± 2°C before to each analysis.

Determination of spreadability

The spreadability of topical gels was measured by glass slide as well as a wooden block system.Accurately about 20 gms of gel sample were placed to the pan and that time was noted.

To calculate the spreadability, the following formula was used

Where,
S= Spreadability
M=Weight tide to upper slide L=Length of glass slide
T=Time taken to separate the slide completely from each other

Escin content

An investigation was conducted to quantify the concentration of the substance within a specificamount of the gel. A sample weighing 1-gram of the gel formulation was placed into a 10 mL flask. Purified water was dropped to the flask, and the mixture was thoroughly shaken until well-mixed. The volume was then adjusted by adding more purified water. The volumetric flask containing the resultant mixture was kept aside for 2 hours. To ensure proper mixing, the flask was shaken on a shaker. Subsequently, the resultant prepared solution was filtered, separating the mixture. The absorbance of the resultant

$$S = \frac{MXL}{T}$$

prepared solution was noted at a fixed wavelength (305 nm) by utilizing a spectrophotometer.

In-vitro escin release study

In-vitro escin permeation studies were executed through cellulose acetate nitrate membraneby utilizing vertical Franz diffusion cells. A cellulose nitrate membrane was placed between the donor and receiver compartments, maintaining the temperature at 37 ± 1°C through a water circulator. The receptor phase, consisting of 12.0 mL of isotonic phosphate buffer (pH7.4) at 37ºC (± 0.5°C), was subjected to continuous stirring at 800 rpm. At predetermined intervals (2, 4, 6, and 24 hours), 0.3 mL escin emugel were extracted from the receptor phase and concurrently substituted with an equivalent volume of fresh medium to uphold volume constancy. These obtained samples underwent analysis via an in-house UV spectroscopy technique to compute the total accumulated quantity of escin.

Stability studies of escin emulgel system

The effects of temperature and humidity on the chemical and physical stability of the newly formulated topical escin emulgel system were evaluated using HDPE containers, following the International Council for Harmonization (ICH) guidelines. Samples of the emulgel were placed in an HDPE container and subjected to three different storage conditions:40°C (Cool condition), 27°C/75% RH (Room Temperature condition), and 40°C/75% RH (Accelerated Condition). These prepared emulgel samples were kept in stability chambers (Thermo lab, India) equipped with precise temperature and humidity control for a duration of 3 months. Samples were retrieved from storage at predetermined intervals spanning 3 months for analysis. The assessment encompassed the examination of active content and essential physiochemical parameters.

RESULT AND DISCUSSION

Preformulation Studies

Escin, with a reported melting point range of 156 to 162°C and an actual melting point of 160°C. This information offers insights into the reported values' accuracy and alignment with experimental results for the specified active ingredient. Escin's has good solubility in diverse solvents, including water, ethanol, and methanol. This show cases escin's adaptable solubility in different chemical settings, implying its potential usefulness in applications demanding dissolution in these solvents.

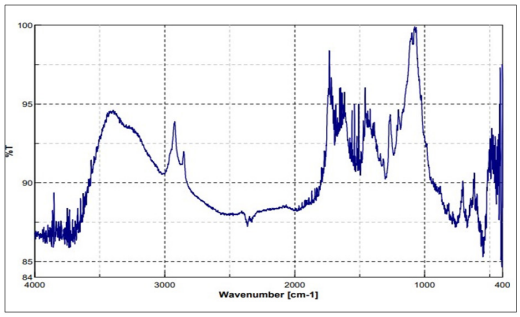

Figure 4: FTIR Spectra of Escin

Escin's infrared spectroscopy reveals the presence of CH_3 groups at (2854 cm) and C=O at (1465 cm). Escin FTIR results show in Figure 4 absorption peaks similar to those in reference IR spectrum values. These peaks belong to a certain functional group. The functional groups displayed by IR spectra accurately match the functional groups of the escin's structural constituents. This result led to the conclusion that the sample medicine provided was pure escin.

Standard calibration curve study

A solution of standard was formulated by dissolving 100 mg of pure escin in 100 mL of distilled water, resulting in a strength of 1-mg/mL. This initial solution was then used to prepare working standard solutions with escin strength of 30.00, 40.00, 50.00, 60.00, 70.00, 80.00, 90.00, 100.00 µg/mL through further dilution with distilled water. Absorbance measurements for all spectral readings were carried out using the Shimadzu 1800 UV-visible spectrophotometer at 305 nm against a blank solution Table 2, Figure 5.

UV Analytical Method of Escin in Phosphate Buffer

Evaluation of physiochemical properties of emulsion and emulgel system

Escin and other required excipients were evaluated for compatibility studies by assessing microscopic and freeze-thaw cycle studies. In microscopic studies primarily change in color,

Table 2: Calibration curve of Escin

Conc.(µg/mL)	*Absorbance (nm)*
30	0.111
40	0.131
50	0.199
60	0.204
70	0.247
80	0.301
90	0.345
100	0.406

transparency, homogeneity and phase separation parameters were checked. Results of physiochemical properties were reported in Table 3.

The homogeneity test of emulgel was performed by visual analysis after the emugels have been stable in the container. Results of physiochemical properties were reported in Tables 4 and 5.

Photo-microscopy

The prepared optimized VL-04 formulation batch showed good consistency. The presence of spherical globules was indicated the formulation of emulsion in gel base, on the basis of photo microscopic evaluation. This indicates the method was successfully employed in the preparation of the emulgel.

Based on the preformulation study, freeze-thaw cycle study and important physiochemical studiesfollowed by assay content in developed gel formulation, the optimization of ingredients was finalized (Figure 6).

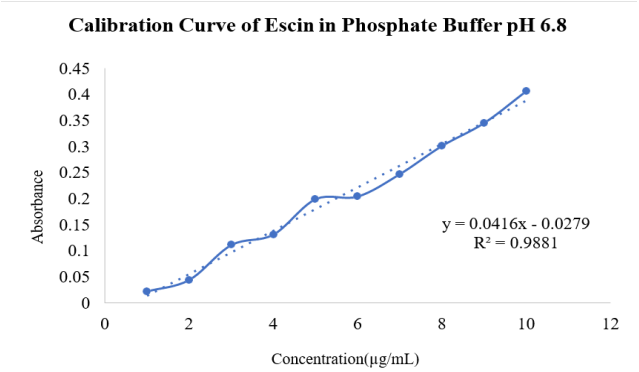

Figure 5: Calibration Curve detection of Escin at 306 nm

Table 3: Physiochemical properties of Escin Emulgel system

Sr.no	*Parameter*	*Specification*	*Observation*
1	Description	Light brown to brown color	Brown coloured gel
2	Odor	Odourless to pungent smell	Pungent smell
3	Texture	Must be homogenous	Homogenous

Table 4: Homogenity of escin emulgel system

Batch	*Change in Color*	*Transparency*	*Phase Separation*	*Homogeneity*
VL-01	No change	Brown color	Phase separation Occurs	Non-homogenous
VL-02	No change	Brown color	Phase separation Occurs	Non-homogenous And grittiness
VL-03	No change	Brown color	No phase separation Occurs	Non-homogenous
VL-04	No change	Brown color	No phase separation Occurs	Non-homogenous

pH

pH of every emulgel batch was analyzed to confirm the cream's in-vitro non-irritating nature. Typically, the skin's pH falls within 5.0 to 7.0, and any value within this range is suitable for emulgel formulation. Consequently, it was inferred that the cream is non-irritating to the skin in an in-vitro context.

Determination of viscosity

Viscosity refers to a formulation's resistance against flow, evaluated through shear. In semisolid formulations meant for prolonged skin contact, elevated viscosity is essential. However, excessive viscosity renders the formulation rigid, hampering spreadability. Thus, a balanced selection of excipients and formulation is crucial for achieving suitable viscosity. The emulgel's viscosity was influenced by both the gelling agent (Carbopol 980) quantity and components within the oily phase. An increment in their amounts correspondsto an increase in emulgel viscosity, results given in Table 6.

Determination of spreadability

Emulgel's spreadability holds significant aesthetic value, influencing the cream's user-friendliness. The Brookfield

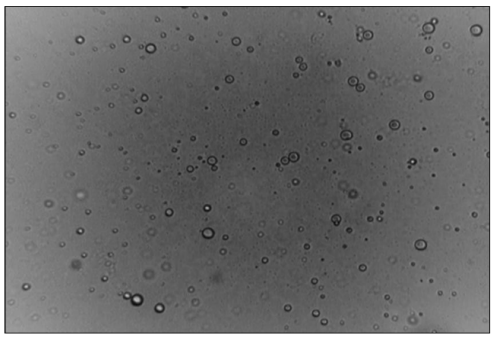

Figure 6: Photo microscopy of VL-04 optimized batch identifying the critical quality attributes (CQAs) through risk analysis

Table 5: Evaluation parameters of optimized batch-Vl-04

Sr. No	*Parameter*	*Observation*
1	Description	Brown colored smooth gel
2	Change in color	Stable
3	Transparency	Brown
4	Phase reparation	No phase separation
5	Homogeneity	Homogenous
6	Viscosity	4462 cps
7	pH	5.3
8	Stability	Stable at room temp

Table 6: Evaluation of phytoconstituents escin emulgel system

Sr. No	*Formulation Batches*	*pH*	*Viscosity (Cps)*	*Spreadability (Hardness) G*
1	VL-01	5.3	8218	1.8
2	VL-02	5.4	6550	2.1
3	VL-03	5.5	4824	1.6
4	VL-04	5.3	4456	1.4

texture analyzer assesses formulation hardness, reflecting its cohesiveness; greater hardness implies reduced spreadability. The dilution potential of the prepared emulgel was investigated to determine its capability to be diluted with the external phase of the system without undergoing phase separation. In freeze-thaw cycle study, spreadability study, irritancy study, dilution test, centrifugal test and consistency test were employed to check stability of the formulation in worst temp and humidity conditions. Having evaluated all the parameters, formulation batch VL-04 was found satisfactory and optimized for further studies. Results of spreadability and freeze-thaw cycle were reported in Table 7.

Escin content

The escin content of the prepared emugel formulation was depicted in Table 8 %Escin content of a proposed emulgel was noted to be 99.45%.

In-vitro escin release study

In *in-vitro* study it is found that, the percentage cumulative drug release of all prepared escin based topical emulgel formulations ranges between 70 to 95% (Table 9). It is also found that the maximum drug release from the optimized VL-04 batch 94.63% at the end of 12 hours. The maximum release is because the optimized formulation consists of the right blend of excipients and surfactant soya lecithin, which is commonly used as a solubility enhancer and for better permeation of poorly water-soluble molecules. The presence of soya lecithin, which is the differentiating point for the optimized VL 04 batch since it acts, shows maximum improvement in escin release compared with other formulations. The optimized VL-04 formulation was compared with other batches based on cumulative escin release, and it is found that cumulative% of escin release of VL-03 was 69% at the end of 12 hours, where a cumulative percentage of escin release of optimized VL-04 batch was found to be 94.63% atthe end of 12 hours which is depicted in Figures 7 and 8.

VL04 Stability studies of escin emulgel system

Stability studies offer insight into the evolution of a escin quality over time, influenced by a spectrum of environmental elements, including temperature, humidity, and light (Table 10). These studies facilitate the determination of optimal storage conditions and the establishment of a suitable shelf life for the substance. As per ICH guidelines, accelerated stability study was performed on prepared optimized-based emulgel. For the stability study, prepared emulgel was filled in PET Jars of 100gm capacity and put into the stability chamber, where the temperature was set at 40°C and relative humidity was set at 75%. Formulations obtained parameters was compared at room temperature and elevated temperature, where optimized formulation was found to stable at room temperature condition.

While at accelerated temp and humidity conditions, pH Viscosity and %drug release were slightly reduced. The increased temp and humidity results in the breakdown of the viscosity of carbomer 980 also leads to the generation of acidic environment within the formulation where this was seen in reduced pH value where initial value 5.3 changed to 4.2 at the completion of 3 months. Reduced viscosity and pH value highlight small deterioration of product quality parameters, suggesting ideal storage condition is room temperature for developed emulgel preparation.

Table 7: Spreadability and the freeze-thaw cycle of escin emulgel system

Sr. No	*Parameter*	*Batch VL-01*	*Batch VL-02*	*Batch VL-0 3*	*Batch VL-04*
1	Spreadability	1.3-1.9	1.4-2.1	1.5-1.8	1.1-1.5
2	Irritancy Test	Non-irritant	Irritant	Non-irritant	Non-irritant
3	Dilution Test	Dilute with Water (10 Times	Dilute with Water (10 Times	Dilute with Water (10 Times	Dilute with Water (10 Times
4	Centri fugal Test	Non Homo genous gel	Non Homo genous gel	Homog enous gel	Homog enous gel
5	Consis tency Test	Thick Semi-Solid Gel	Semisolid Gel	Semisolid Gel	Semisolid Gel

Table 8: Escin content of the emugel

Sr. No	*Batch Code*	*Escin Content %*
1	VL 04	99.45%

SUMMARY

Aesculus hippocastanum or horse chestnut extract containing escin possessing anti-inflammatory, anti-edema activity and venotonic were successfully added into topical gel system for the management of varicose veins. Proposed work is carried out to develop a novel escin emulgel system to treat varicose veins. Scientifically proven ingredients were combined for unmet need of the management of varicose veins. An endeavor has been undertaken to create a hybrid drug delivery system

Table 9: *In-vitro* escin release profile of emulgel system

Time in hr.	*VL01*	*VL02*	*VL03*	*VL04*
1	4.25	3.025	6.01	6.15
2	10.65	5.12	8.12	12.25
3	18.98	11.58	14.02	18.65
4	22.35	20.78	18.01	26.48
5	30.45	24.04	23.11	36.4
6	36.45	30.34	27	42.22
7	42.05	34.05	36.04	55.12
8	54.54	41.98	42.09	62.49
9	62.32	48.25	49.03	74.15
10	73.15	61.25	56	80.59
11	80.06	74.52	62.12	85.02
12	85.88	79.25	69	94.63

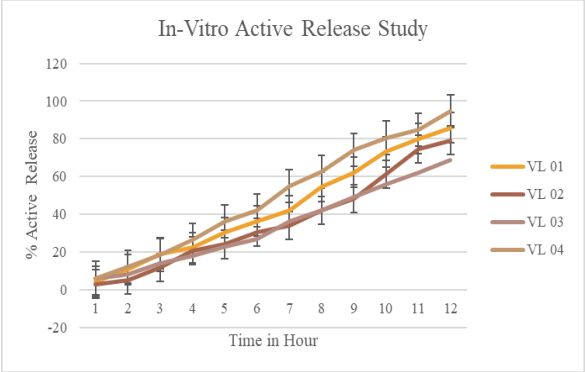

Figure 7: *In-vitro* percentage escin release of escin emulgel

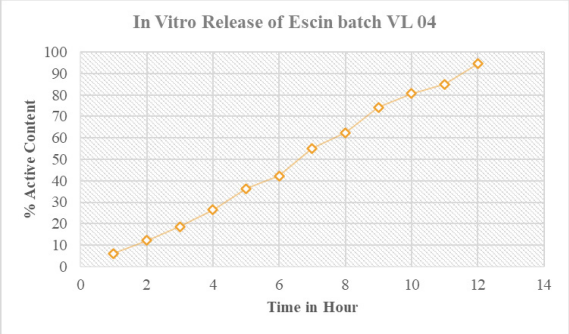

Figure 8: *In-vitro* percentage escin release of emulgel formulation batch

by merging two distinct delivery mechanisms: emulsions and aqueous carbomer gel. This novel approach addresses the limitations inherent in traditional therapies for treating varicose veins. The UV spectral analysis of escin in conducted at various concentrations in phosphate buffer with a pH of 6.8 demonstrated a linear outcome. Hence it obeyed Beer's Lambert law. Homogenous emulsions of escin system were composed using a high-speed homogenizer mixer. Total 4 trial emulgel formulations were composed by keeping drug (escin), phenoxyethanol, propylene glycol, and liquid paraffin as fixed proportion and differing stearic acid and carbomer 980 concentrations. For batches, batch VL-04 (Table 11) has the optimized formula finalized after a thorough physicochemical and analytical investigation. The developed emulgel formulations were characterized for escin in content, viscosity, pH spreadability studies and *in-vitro* escin in release study. The presence of spherical globules was indicated the formulation of emulsion in gel base on the basis of photo microscopic evaluation. Physiochemical evaluation studies such as Freeze-thaw cycle, pH, viscosity determination, and spreadability are within the acceptable range, thus provided evidence to optimize the formulation. The characterization studies provided good stability of developed topical gel system which can be good basis for scale up of product. As per the *in-vitro* escin in release studies, optimized emulgel formulation (VL 04) was transformed into emulgel formulation with the addition carbomer 980 gel *in-vitro* escin in release study of all escin emulgel formulation was conducted by diffusion method. Out of all the emulgel formulated versions, the optimized

Table 10 Stability study Report of Escin based emulgel- Batch No-VL 04 (Condition: 27°C and 75% RH (Room temperature)

		Quality Control Parameters				
Formulation Batch	*Months*	*Appearance*	*pH (Mean Value)*	*Viscosity (Mean Value) in cPs*	*%Active Content (Mean Value) (%)*	*In-vitro Active Release (Mean Value) (%)*
Batch-VL 04	0	Brown Colored Smooth Gel	5.3	4462	99.45	94.63
	1	Brown Colored Smooth Gel	5.5	4354	98.14	92.55
	3	Brown Colored Smooth Gel	5.8	4320	97.58	93.05

Table 11: Stability study Report of Escin based emulgel- Batch No-VL 04 (Condition: 40°Cand 75% RH)-Accelerated Condition)

		Quality Control Parameters				
Formulation Batch	*Months*	*Appearance*	*pH (Mean Value)*	*Viscosity (Mean Value) in cPs*	*%Active Content (Mean Value) (%)*	*In-vitro Active Release (Mean Value) (%)*
Batch-VL 04	0	Brown Colored Smooth Gel	5.3	4462	99.45	94.63
	1	Brown Colored Smooth Gel	4.5	4125	97.25	88.15
	3	Brown Colored Smooth Gel	4.2	3825	95.14	85.24

formulation (VL04) exhibited a controlled and prolonged drug release of 94.63 ± 0.22% over a span of 12 hours. The presence of soya lecithin, which is the differentiating point for the optimized B4 batch since it acts, shows maximum improvement in escin release compared to other formulations.

Developed emulgel formulation batch VL04 was described for viscosity, pH, spreadability, escin content and *in-vitro* escin release study. The pH of the proposed emulgel was found to be 5.3 and it was noted to be brown colored dispersion. Escin content was found to be 99.45%. The cumulative percentage of Escin release of optimized B4 batch was found to be 94.63% at the end of 12 hours. The viscosity for the optimized escin emulgel was noted to be satisfactory. A stability study suggests ideal storage condition of escin based emulgel system is "Store at room temperature, protect from direct sunlight".

CONCLUSION

It is concluded that the escin-enriched topical emulgel formulation system demonstrated promising stability and consistent drug release characteristics throughout freeze-thaw cycles. The formulation exhibited good stability under the tested conditions and maintained its intended drug release profile. These findings suggest that the freeze-thaw process did not significantly impact the escin- enriched topical gel formulation's overall quality, stability, or drug release behavior, highlighting its potential for further development as a viable pharmaceutical product. Thus, the current study presents a novel approach, emphasizing the safety, effectiveness, and potential of the formulated escin-based emulgel as a promising solution for topical managing varicose veins. However, additional confirmation through experimental and clinical investigations is crucial to validate the findings of this study.

ACKNOWLEDGEMENT

The authors are very much thankful to the Principal and Management of Ashokrao Mane College of Pharmacy, Peth Vadgaon for providing the required facilities for research work.

REFERENCES

1. Villalta SB, Bagatella P, Piccioli A, Lensing A, Prins MH, Prandoni P. Assessment of validity and reproducibility of a clinical scale for the post-thrombotic syndrome. Haemostasis. 1994;24(suppl 1):158a.
2. Raju S. Best management options for chronic iliac vein stenosis and occlusion. Journal of vascular surgery. 2013 Apr 1;57(4):1163-9.
3. Davies AH. Re:'Management of Chronic Venous Disease: Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS)'. European Journal of Vascular and Endovascular Surgery. 2016 Jan 1;51(1):156.
4. Coleridge-Smith P, Labropoulos N, Partsch H, Myers K, Nicolaides A, Cavezzi A. Duplex ultrasound investigation of the veins in chronic venous disease of the lower limbs–UIP Consensus Document. Part I: Basic principles. Phlebology. 2006 Dec 1;21(4):158-67.
5. Auvert JF, Chleir F, Coppé G, Hamel-Desnos C, Moraglia L, Pichot O. Quality standards for ultrasound assessment of the superficial venous system of the lower limbs. Report of the French Society for Vascular Medicine. Journal des Maladies Vasculaires. 2014 Jan 18;39(1):26-46.
6. Labropoulos N, Tiongson J, Pryor L, Tassiopoulos AK, Kang SS, Mansour MA, Baker WH. Definition of venous reflux in lower-extremity veins. Journal of vascular surgery. 2003 Oct 1;38(4):793-8.
7. Abai B, Labropoulos N. Duplex ultrasoundscanning for chronic venous obstruction and valvular incompetence. InHandbook of Venous Disorders: Guidelines of the American Venous Forum Third Edition 2008 Dec 26 (p. 142). Boca Raton: CRC Press.
8. Quarto G, Genovese G, Apperti M, Amato B, Benassai G, Furino E. Is the fibrotic parietal thickening a reliable parameter for diagnosing previous asymptomatic deep vein thrombosis. Ann Ital Chir. 2015 Jun;86:427-31.
9. Neglen P, Raju S. A comparison between descending phlebography and duplex Doppler investigation in the evaluation of reflux in chronic venous insufficiency: a challenge to phlebography as the "gold standard". Journal of vascular surgery. 1992 Nov 1;16(5):687-93.
10. Labropoulos N, Borge M, Pierce K, Pappas PJ. Criteria for defining significant central vein stenosis with duplex ultrasound. Journal of Vascular surgery. 2007 Jul 1;46(1):101-7.
11. Pichot O, Menez C. Role of duplex ultrasound investigation in the management of postthrombotic syndrome. Phlebolymphology. 2016 Jan 1;23(2):102-11.
12. Spencer EB, Stratil P, Mizones H. Novel treatment techniques for recanalization of femoral-popliteal deep venous occlusion from chronic thrombosis. Techniques in Vascular and Interventional Radiology. 2014 Jun 1;17(2):114-20.
13. Maleti O, Perrin M. Reconstructive surgery for deep vein reflux in the lower limbs: techniques, results and indications. European Journal of Vascular and Endovascular Surgery. 2011 Jun 1;41(6):837-48.
14. Pavlović MD, Schuller-Petrović S, Pichot O, Rabe E, Maurins U, Morrison N, Pannier F. Guidelines of the first international consensus conference on endovenous thermal ablation for varicose vein disease–ETAV consensus meeting 2012. Phlebology. 2015 May;30(4):257-73.
15. Mahnken AH, Thomson K, de Haan M, O'Sullivan GJ. CIRSE standards of practice guidelines on iliocaval stenting. Cardiovascular and interventional radiology. 2014 Aug;37:889-97.
16. Abudayeh ZH, Al Azzam KM, Naddaf A, Karpiuk UV, Kislichenko VS. Determination of four major saponins in skin and endosperm of seeds of horse chestnut (Aesculus hippocastanum L.) using high performance liquid chromatography with positive confirmation by thin layer chromatography. Advanced pharmaceutical bulletin. 2015 Nov;5(4):587.
17. Sanjivkumar B, Rajkumar D, Mallikarjun P, Karankumar B, Rao KS. Development and method validation of Aesculus hippocastanum extract. Int. Res. J. Pharm. 2012;3:324-8.
18. Dar RA, Shahnawaz M, Qazi PH. General overview of medicinal plants: A review. The journal of phytopharmacology. 2017;6(6):349-51.
19. BK PP, Kumar P. Clinical Study of varicose veins and their management. International Journal of Biomedical and Advance Research. 2015 Aug 30;6(8):564-8.
20. Kamble KU, Nitave SA. A REVIEW ON VARICOSE VEINS AND ITS TREATMENTS.
21. Mor D, Dande P. Varicose veins: an overview of current and herbal treatments. International Journal of Pharmaceutical Sciences and Research. 2017 May 1;8(5):1959.
22. Özkan EE, Mesut B, Polat A, Nur TA. In vitro skin permeation of escin in the new gel formulation of Aesculus hippocastanum (Horse Chestnut). Journal of Faculty of Pharmacy of Istanbul University. 2016 Dec 1;46(2):79-88.
23. Prakash V, Jaiswal NI, Srivastava MR. A review on medicinal properties of Centella asiatica. Asian J Pharm Clin Res. 2017;10(10):69-74.
24. Arora D, Kumar M, Dubey SD. Centella asiatica-A Review of it's Medicinal Uses and Pharmacological Effects. Journal of Natural remedies. 2002 Jun 1;2(2):143-9.
25. Ma ZF, Zhang H. Phytochemical constituents, health benefits, and industrial applications of grape seeds: A mini-review. Antioxidants. 2017 Sep 15;6(3):71.
26. Loveleen Preet Kaur TK. Topical Gel: A Recent Approach for Novel Drug delivery. Asian Journal Of Biomedical &

Pharmaceutical Sciences, e-ISSN: 22 (3 (17)), 1. 2013;5.
27. Mulla SA, Pai S. Varicose veins: a clinical study. International Surgery Journal. 2017 Jan 25;4(2):529-33.
28. Dudek-Makuch M, Studzińska-Sroka E. Horse chestnut-efficacy and safety in chronic venous insufficiency: an overview. Revista Brasileira de Farmacognosia. 2015 Oct;25(5):533-41.
29. Zelbienė E, Draksiene G, Savickas A, Kopustinskiene D, Masteikova R, Bernatoniene J. In vitro permeation studies of phenolics from horse chestnut seed gels prepared with different polyacrylic acid polymer derivatives. Acta pharmaceutica. 2015 Jun 30;65(2):181-9.
30. Corciova A, Ivanescu B. Comparative quantification of escin from different products. European Chemical Bulletin. 2017;6(9):400-4.
31. Sultan RA, Mahmood ZA, Azhar I, Hasan MM, Ahmed S. Pharmacognostic and phytochemical investigation of aerial parts of Centella asiatica Linn. International Journal of Phytomedicine. 2012;4(1):125-33.
32. Wilkinson JA, Brown AM. Horse Chestnut–Aesculus hippocastanum: Potential applications in cosmetic skin-care products. International journal of cosmetic science. 1999 Dec;21(6):437-47.
33. Tomečková V, Rehák̆ová M, Mojžišová G, Wadsten T, Zelenáková K, Komanický V. Spectral study of modified natural clinoptilolite with pharmacologically active escin and horse chestnut extract. Spectroscopy Letters. 2016 Feb 7;49(2):63-72.
34. Patlolla JM, Qian L, Biddick L, Zhang Y, Desai D, Amin S, Lightfoot S, Rao CV. β-Escin inhibits NNK-induced lung adenocarcinoma and ALDH1A1 and RhoA/Rock expression in A/J mice and growth of H460 human lung cancer cells. Cancer Prevention Research. 2013 Oct 1;6(10):1140-9.
35. Suryavanshi SV, Kulkarni YA. Attenuation of cardiac autonomic neuropathy by escin in diabetic rats. Pharmacology. 2021 Mar 19;106(3-4):211-7.
36. Elmas O, Erbas O, Yigitturk G. The efficacy of Aesculus hippocastanum seeds on diabetic nephropathy in a streptozotocin-induced diabetic rat model. Biomedicine & Pharmacotherapy. 2016 Oct 1;83:392-6.
37. Kimura H, Ogawa S, Katsube T, Jisaka M, Yokota K. Antiobese effects of novel saponins from edible seeds of Japanese horse chestnut (Aesculus turbinata BLUME) after treatment with wood ashes. Journal of agricultural and food chemistry. 2008 Jun 25;56(12):4783-8.
38. WU SJ, SA RN, GU ZR, Zhao P, YU J, Wang YH, GE B. The hepatoprotective effect of Aesculus hippocastanum seed extract against concanavalin A-induced acute liver injury in mice via inhibition of ROS and JNK pathway. Chinese Pharmacological Bulletin. 2018:412-8.
39. ERTÜRK Ö. Antibacterial and antifungal effects of the leaf, seed, seed coat and fruit capsule of Aesculus hippocastanum (Sapindaceae) extracts. Acta Biologica Turcica. 2017 Feb 10;30(1):20-3.
40. Belcaro G, Rosaria Cesarone M, Ledda A, Cacchio M, Ruffini I, Ricci A, Ippolito E, Di Renzo A, Dugall M, Corsi M, Marino Santarelli AR. O-(β-hydroxyethyl)-rutosides systemic and local treatment in chronic venous disease and microangiopathy: an independent prospective comparative study. Angiology. 2008 Feb;59(1_suppl):7S-13S.
41. Matsuda H, Li Y, Murakami T, NINOMIYA K, YAMAHARA J, YOSHIKAWA M. Effects of escins Ia, Ib, IIa, and IIb from horse chestnut, the seeds of Aesculus hippocastanum L., on acute inflammation in animals. Biological and Pharmaceutical Bulletin. 1997 Oct 15;20(10):1092-5.
42. Maghsoudi H, Hallajzadeh J, Rezaeipour M. Evaluation of the effect of polyphenol of escin compared with ibuprofen and dexamethasone in synoviocyte model for osteoarthritis: an in vitro study. Clinical rheumatology. 2018 Sep;37:2471-8.
43. Ruffini, I., Belcaro, G., Cesarone, M., Dugall, M.(2004) Efficacy of topical treatment with aescin essential phospholipids gel in venous insufficiency and hypertension. Angiology.55 Suppl 1:S19-21.
44. Ogawa S, Kimura H, Niimi A, Katsube T, Jisaka M, Yokota K. Fractionation and structural characterization of polyphenolic antioxidants from seed shells of Japanese horse chestnut (Aesculus turbinata BLUME). Journal of agricultural and food chemistry. 2008 Dec 24;56(24):12046-51.
45. Pietta P, Mauri P, Facing RM, Carini M. High-performance liquid chromatographic analysis of β-escin. Journal of Chromatography A. 1989 Jan 1;478:259-63.
46. Paterska M, Bandurska H, Wysłouch J, Molińska-Glura M, Moliński K. Chemical composition of horse-chestnut (Aesculus) leaves and their susceptibility to chestnut leaf miner Cameraria ohridella Deschka & Dimić. Acta physiologiae plantarum. 2017 Apr;39:1-6.
47. Baraldi, C., Bodecchi, L. M., Cocchi, M., Durante, C., Ferrari, G., Foca, G., and Ulrici, A. (2007). Chemical composition and characterisation of seeds from two varieties (pure and hybrid) of Aesculus hippocastanum. Food chem., 104(1):229-236.
48. Sirtori CR. Aescin: pharmacology, pharmacokinetics and therapeutic profile. Pharmacological Research. 2001 Sep 1;44(3):183-93.
49. Mukherjee PK, Harwansh RK, Bahadur S, Banerjee S, Kar A. Evidence-based validation of Indian traditional medicine: Way forward. InFrom Ayurveda To Chinese Medicine 2017 (pp. 137-167).
50. Gautam V, Raman RM, Kumar A. Exporting Indian Healthcare: Export Potential of Ayurveda and Siddha Products and Services. Export Import Bank of India; 2003.
51. Lichota A, Gwozdzinski L, Gwozdzinski K. Therapeutic potential of natural compounds in inflammation and chronic venous insufficiency. European Journal of Medicinal Chemistry. 2019 Aug 15;176:68-91.
52. Gallelli L. Escin: a review of its anti-edematous, anti-inflammatory, and venotonic properties. Drug design, development and therapy. 2019 Sep 27:3425-37.