RESEARCH PAPER

Formulation, Optimization & In-Vitro Evaluation of Quercetin-Phytol Cream For Potential Application In Irritant Contact Dermatitis

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ABSTRACT

Background: Inflammatory skin conditions are associated with irritation, redness, discomfort and disruption of the normal skin barrier. Topical drug delivery provides an important approach for localized management of inflammatory skin conditions by delivering the active constituents directly to the affected site. Natural bioactive compounds have gained considerable interest because of their potential anti-inflammatory and antioxidant properties. Quercetin is a naturally occurring flavonoid reported to possess antioxidant and anti-inflammatory activities, whereas phytol is a lipophilic diterpene alcohol with reported biological activities. However, formulation-related limitations and limited investigation of their combined topical application warrant further pharmaceutical evaluation. **Objective:** The aim of the present study was to prepare and evaluate a Quercetin and Phytol based topical cream and to study its physicochemical properties, in-vitro diffusion behaviour and preliminary anti-inflammatory activity. **Methods:** Seven preliminary trial formulations (**F1–F7**) were developed by maintaining the selected quantities of the active ingredients while varying the concentrations of selected excipients involved in the cream base and structuring system. The formulations were evaluated for their relevant physicochemical characteristics, and the formulation showing comparatively satisfactory overall performance was selected for further development. Based on the trial formulation results, **F5 was selected as the optimized formulation**, and a final 30-g batch was prepared. The prepared cream was evaluated for appearance, homogeneity, pH, spreadability, viscosity, extrudability and other applicable formulation characteristics. In-vitro diffusion of the optimised formulation was studied using Franz diffusion system. The preliminary anti inflammatory activity was evaluated by protein denaturation and HRBC membrane stabilisation assays. The experimental data were subjected to appropriate statistical analysis. **Results:** The optimized Quercetin–Phytol cream demonstrated satisfactory physicochemical characteristics and showed progressive diffusion during the study period. The formulation exhibited concentration-dependent inhibition in the protein denaturation assay and concentration-dependent membrane stabilization in the HRBC assay. The optimized formulation showed **68.40% inhibition of protein denaturation at 100 µg/mL** and **64.50% HRBC membrane stabilization at 100 µL/mL** under the experimental conditions. The recalculated IC_{50} value for the protein denaturation assay was approximately **72.09 µg/mL**. **Conclusion:** The findings of the present investigation indicate that the developed Quercetin–Phytol cream possesses satisfactory pharmaceutical characteristics and demonstrates promising preliminary in-vitro anti-inflammatory activity. The combination of Quercetin and Phytol in a topical cream therefore warrants further investigation to establish its potential application in inflammatory skin conditions.

Keywords: Quercetin, Phytol, topical cream, anti-inflammatory activity, protein denaturation, HRBC membrane stabilization, Franz diffusion.

1. INTRODUCTION

Inflammation is a complex biological response that occurs in response to tissue injury, irritation, infection and other pathological stimuli. Inflammatory skin conditions may be associated with redness, irritation, swelling, discomfort and impairment of the normal protective function of the skin. Topical drug delivery represents an important approach for the management of localized skin conditions because it allows active pharmaceutical or bioactive constituents to be delivered directly to the site of application. [1,2]

Inflammation is treated with a wide range of conventional anti-inflammatory agents, including corticosteroids and non-steroidal anti-inflammatory drugs. Although these agents can provide effective control of inflammation, prolonged or inappropriate topical use may be associated with limitations such as irritation, skin atrophy and other local adverse effects. Consequently, increasing attention has been directed towards naturally occurring compounds with potential anti-inflammatory and antioxidant properties. [3,4]

Quercetin is a naturally occurring flavonoid widely distributed in various plants and dietary sources. It has been investigated for its antioxidant and anti-inflammatory properties and has attracted considerable interest as a

potential bioactive pharmaceutical ingredient. However, its pharmaceutical utilization in topical formulations may be influenced by physicochemical limitations, particularly poor aqueous solubility and restricted delivery through the skin. [5,6]

Phytol is a naturally occurring diterpene alcohol and an important component of chlorophyll-derived compounds. It possesses a lipophilic nature and has been investigated for various biological activities. Its incorporation into a topical formulation containing Quercetin provides an approach for developing a formulation in which both bioactive constituents can be delivered through a suitable topical dosage form. [7,8]

The formulation of a topical cream requires appropriate selection of the oil phase, aqueous phase, emulsifying agents, consistency-enhancing agents, humectants and other excipients to obtain acceptable physicochemical properties and formulation stability. Therefore, systematic formulation trials are necessary to identify an appropriate combination of excipients capable of producing a satisfactory cream formulation. [9,10]

In the present investigation, seven trial formulations (F1–F7) were developed by maintaining the selected active ingredient quantities while modifying selected excipient concentrations. The trial formulations were evaluated to identify a formulation with satisfactory physical and pharmaceutical characteristics. Based on the results obtained during formulation trials, F5 was selected for further development, followed by preparation of the final optimized 30-g Quercetin–Phytol cream.

The optimized formulation was subsequently evaluated using relevant pharmaceutical evaluation parameters. The in-vitro diffusion was studied by Franz diffusion system and preliminary anti-inflammatory activity was studied by protein denaturation and HRBC membrane stabilisation assays.These approaches were selected to provide complementary information regarding the pharmaceutical performance and preliminary biological activity of the developed formulation. [11–13]

Aim of the Study

"Therefore, the present study aimed to develop and evaluate a Quercetin–Phytol topical cream and investigate its physicochemical characteristics, in-vitro diffusion behaviour and preliminary anti-inflammatory activity using protein denaturation and HRBC membrane stabilization models.".

2. MATERIALS AND METHODS

2.1 Materials

Quercetin and phytol were used as the active ingredients for the development of the topical cream. Stearic acid, cetyl alcohol, liquid paraffin, Span-20, Tween-80, glycerin, propylene glycol and methyl paraben were used as formulation excipients. Purified water was used for preparation of the aqueous phase. The active ingredients were obtained from **Yucca Enterprises, Mumbai**, while the formulation excipients were obtained from the college laboratory. The Certificate of Analysis (COA) of phytol reported a purity of 90% by HPLC and confirmed its identity by TLC.

2.2 Formulation Development

Seven preliminary trial formulations (F1–F7) were prepared to identify a suitable composition for the Quercetin–Phytol cream. The quantities of the active ingredients were maintained according to the formulation design, while selected concentrations of the formulation excipients were varied during the trial batches. The prepared formulations were evaluated for their physical and pharmaceutical characteristics. Based on the comparative evaluation of the trial formulations, F5 was selected as the optimized formulation and was subsequently used for preparation of the final batch.

Systematic variation of excipients during formulation development and evaluation of the resulting cream characteristics was conducted according to general principles of semisolid dosage-form formulation and development . [14,15]

2.3 Preparation of Quercetin–Phytol Cream

The optimized formulation was prepared as a 30-g batch using the conventional emulsification method. The composition of the final formulation included Quercetin, Phytol, stearic acid, cetyl alcohol, liquid paraffin, Span-20, Tween-80, glycerin, propylene glycol, methyl paraben and purified water. The preparation involved separate oil and aqueous phases followed by emulsification under controlled temperature and continuous stirring. [14,15]

2.3.1 Preparation of Oil Phase

Stearic acid, cetyl alcohol and liquid paraffin were accurately weighed and transferred into a clean, dry beaker. Span-20 and phytol were added to the oil phase. The mixture was heated on a hot plate at 70–75°C with gentle stirring until a clear and homogeneous oily phase was obtained.

2.3.2 Preparation of Aqueous Phase

In a separate beaker, the required quantity of purified water was taken and Tween-80, glycerin and propylene glycol

were added. Methyl paraben was incorporated and dissolved completely. The aqueous phase was heated to 70–75°C to obtain a temperature comparable with that of the oil phase.

2.3.3 Preparation of Quercetin Dispersion

The required quantity of Quercetin was dispersed in a small quantity of the previously measured propylene glycol using trituration in a glass mortar to obtain a smooth and lump-free dispersion. The dispersion was kept aside and protected from direct exposure to high temperature.

2.3.4 Emulsification and Incorporation of Quercetin

After both phases reached approximately 70–75°C, the aqueous phase was slowly incorporated into the oil phase with continuous stirring. The mixture was stirred for approximately 8–10 minutes to obtain a uniform emulsion. The emulsion was subsequently allowed to cool gradually with continuous stirring. At approximately 40°C, the prepared Quercetin dispersion was incorporated into the emulsion and mixed thoroughly to obtain uniform distribution of the active ingredient. [14,15]

Important: We should not put a separate citation after every individual preparation step. One citation at the end of the overall preparation-method paragraph/section is sufficient because these steps represent the formulation procedure based on standard semisolid formulation principles, while the specific temperatures, times and quantities are your experimental conditions.

2.4 Evaluation of the Optimized Cream

The optimized Quercetin–Phytol cream was evaluated for its relevant physicochemical and pharmaceutical characteristics. The evaluation included appearance, homogeneity, pH, spreadability, viscosity, extrudability and other applicable formulation parameters. The extrudability of the cream was determined using a collapsible tube, with the reported formulation showing 90% extrudability under the specified test conditions. Standard evaluation parameters for semisolid topical preparations were considered during assessment. [14,15]

2.5 In-vitro Franz Diffusion Study

The in-vitro diffusion behavior of Quercetin from the optimized Quercetin–Phytol cream was investigated using a Franz diffusion cell. A cellulose acetate membrane was used as the diffusion membrane, with phosphate-buffered saline (PBS) serving as the receptor medium. The diffusion study was performed at **37 ± 1°C**, and samples were withdrawn at **0, 1, 3, 6, and 24 hours**. The amount of quercetin released was measured using a **UV–visible spectrophotometer at 370 nm**. The Franz diffusion-cell approach for evaluating drug release/permeation across a membrane was based on established in-vitro diffusion methodology. [16]

Your thesis/PPT specifically records these experimental conditions: cellulose acetate membrane, PBS receptor medium, 37 ± 1°C, 0–24 h sampling and UV analysis at 370 nm.

2.6 In-vitro Anti-inflammatory Assays

The preliminary anti-inflammatory activity of the optimized formulation was evaluated using protein denaturation and HRBC membrane stabilization assays. Quercetin, phytol and the Quercetin–Phytol cream were evaluated at different concentrations and compared with the respective standard drugs. The use of complementary in-vitro models was intended to provide preliminary assessment of anti-inflammatory activity through different experimental endpoints. [17,18]

2.6.1 Protein Denaturation Assay

The protein denaturation assay was carried out to assess the ability of the test samples to prevent protein denaturation. Different concentrations of the samples were tested, and the percentage inhibition was calculated for each concentration. The resulting concentration-dependent response was used to compare the anti-inflammatory activity of the test samples. The study was performed following the established method reported by Mizushima and Kobayashi. [17]

2.6.2 HRBC Membrane Stabilization Assay

The HRBC membrane stabilization assay was performed to assess the membrane-stabilizing activity of the test samples. The percentage stabilization was determined at different concentrations and compared with the standard treatment. The assay was based on the established HRBC membrane-stabilization approach used for preliminary screening of anti-inflammatory activity. [18]

Your thesis literature specifically identifies HRBC membrane stabilization as an established in-vitro preliminary screening method and describes its use together with protein denaturation to provide complementary evidence of anti-inflammatory activity.

2.7 Statistical Analysis

The results were expressed as **mean ± standard deviation (SD)**. Appropriate statistical methods were used to compare the results between the experimental groups. A **p-value of less than 0.05 ($p < 0.05$)** was considered statistically significant.

3. RESULTS

3.1 Formulation Development and Selection of F5

Seven preliminary trial formulations (F1–F7) of Quercetin–Phytol cream were prepared to identify a suitable formulation composition. The active ingredients were maintained according to the formulation plan, while selected concentrations of the formulation excipients were varied among the trial batches.

The prepared trial formulations were evaluated for their physical appearance, homogeneity, consistency and general pharmaceutical characteristics. Variation in the concentrations of the formulation excipients resulted in differences in the physical characteristics and handling properties of the prepared creams.

Based on the comparative evaluation of the seven trial formulations, **formulation F5 was selected as the optimized formulation** because it demonstrated comparatively satisfactory physical and pharmaceutical characteristics. The selected formulation was subsequently used for preparation of the final **30-g Quercetin–Phytol cream**.

The optimized formulation contained Quercetin and Phytol as the active ingredients along with stearic acid, cetyl alcohol, liquid paraffin, Span-20, Tween-80, glycerin, propylene glycol, methyl paraben and purified water.

3.2 Evaluation of Optimized Formulation

The optimized Quercetin–Phytol cream was evaluated for its organoleptic and physicochemical characteristics, including appearance, homogeneity, pH, spreadability, viscosity, extrudability and drug content uniformity.

The formulated cream showed a **pale-yellow colour with a pleasant odour and smooth texture**. It appeared homogeneous on visual inspection, suggesting that the formulation ingredients were **uniformly distributed throughout the cream**.

The optimized cream showed a **pH of 5.24**, which was within a suitable range for topical application. The formulation also showed a **spreadability value of 7.69**, indicating that the cream could be easily and uniformly spread over the skin.

The viscosity of the formulation was found to be **5760–5940 cP at 10 rpm**, while a viscosity of **9000 cP at 5 rpm** was observed. The change in viscosity with rotational speed indicated the flow behaviour of the cream and its suitability for topical application.

The extrudability of the formulation was found to be **90%**, indicating satisfactory ease of extrusion from the collapsible tube.

Drug Content Uniformity

The drug content of the optimized Quercetin–Phytol cream was determined by UV-visible spectrophotometric analysis at **370 nm**. The absorbance of the prepared sample was found to be **0.975**, corresponding to a drug content of **97.5%**. The obtained value was within the specified acceptable range of **95–105%**, indicating satisfactory incorporation and uniform distribution of Quercetin within the formulated cream.

Table 1. Evaluation of optimized Quercetin–Phytol cream

Evaluation parameter	Result
Appearance	Pale yellow
Odour	Pleasant
Texture	Smooth
Homogeneity	Homogeneous
pH	5.24
Spreadability	7.69
Viscosity at 10 rpm	5760–5940 cP
Viscosity at 5 rpm	9000 cP
Extrudability	90%

Drug content	**97.5%**

Overall, the optimized Quercetin–Phytol cream demonstrated satisfactory organoleptic and physicochemical properties, including acceptable pH, spreadability, viscosity, extrudability and drug content uniformity.

3.3 In-vitro Franz Diffusion Study

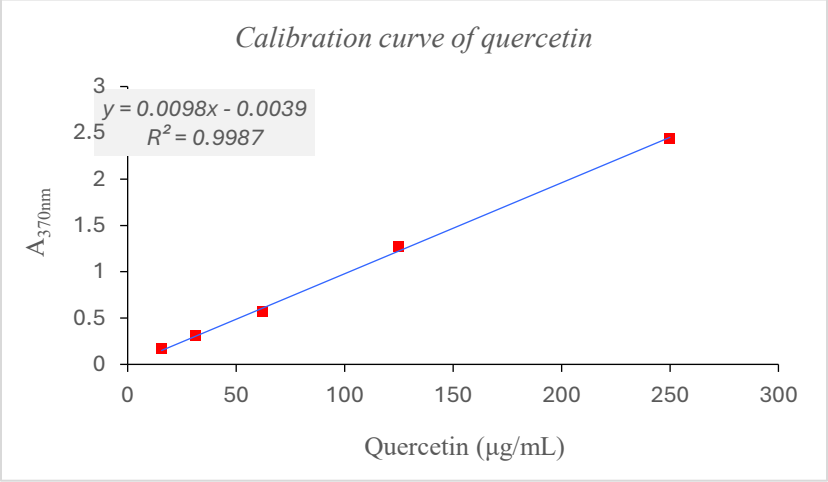
The in-vitro diffusion study was carried out to evaluate the diffusion behaviour of Quercetin from the optimized Quercetin–Phytol cream using a Franz diffusion system. A cellulose acetate membrane was used as the diffusion membrane and phosphate-buffered saline (PBS) was used as the receptor medium. The study was conducted at 37 ± 1°C and samples were collected at predetermined time intervals of 0, 1, 3, 6 and 24 h. Quercetin concentration was determined spectrophotometrically at 370 nm..

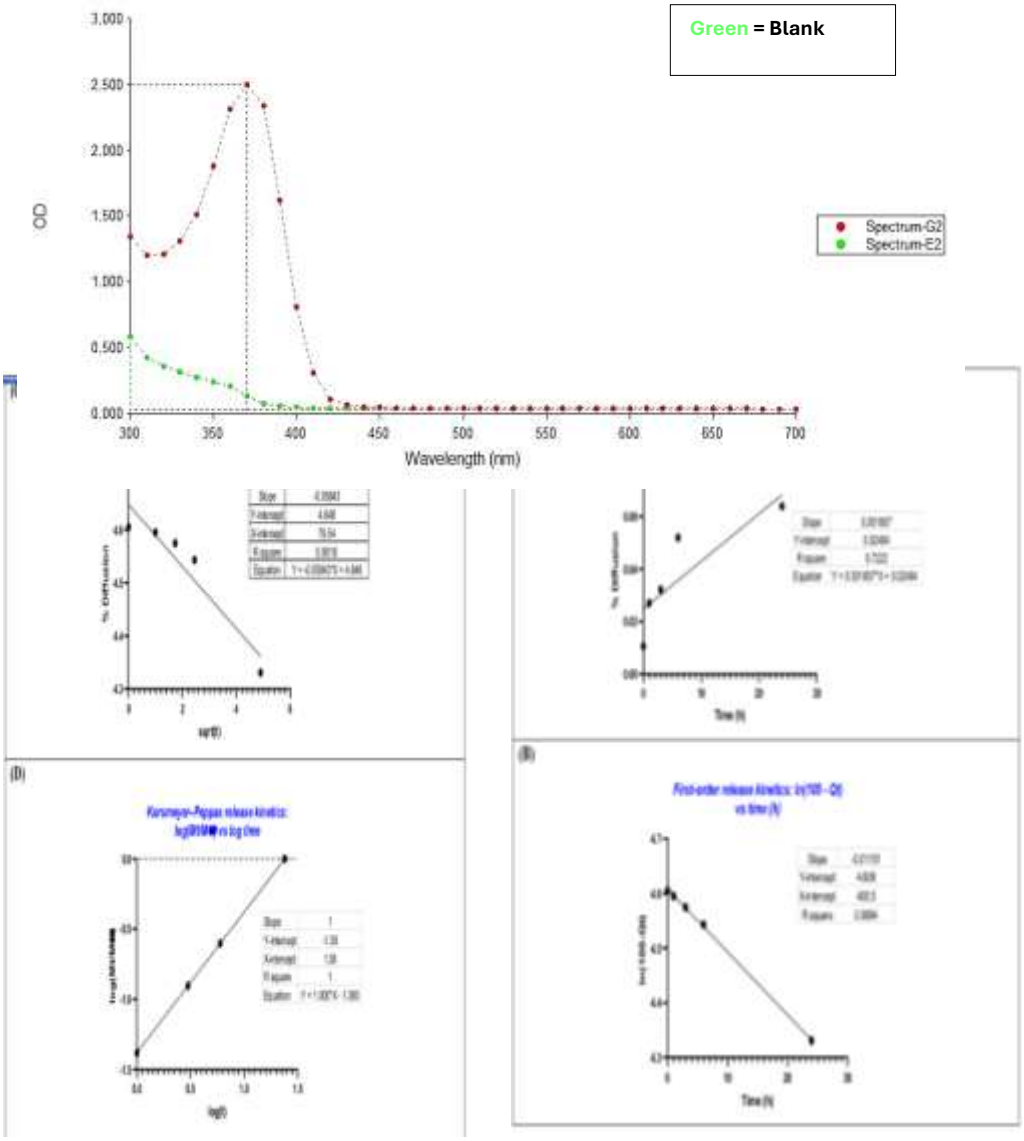
The cumulative percentage diffusion increased progressively with increasing diffusion time. The mean cumulative diffusion was **1.06% at 0 h, 2.70% at 1 h, 3.21% at 3 h, 5.20% at 6 h and 6.39% at 24 h**.

Table 2. In-vitro cumulative diffusion profile of optimized Quercetin–Phytol cream

Time (h)	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean (%)	SD	SE
0	0.7041	0.6020	0.6020	1.06	0.10	0.06
1	2.3367	1.0102	1.5204	2.70	1.12	0.64
3	2.3367	1.9286	1.5204	3.21	0.68	0.39
6	3.5612	3.2551	2.5408	5.20	0.87	0.50
24	3.7653	3.8673	3.8673	6.39	0.10	0.06

The results demonstrated a gradual increase in cumulative Quercetin diffusion throughout the 24-h study period, indicating sustained diffusion of the active ingredient from the cream formulation.





3.4 Protein Denaturation Assay

The protein denaturation assay demonstrated a concentration-dependent increase in inhibition of protein denaturation for Diclofenac sodium, Quercetin, Phytol and Quercetin–Phytol cream.

Diclofenac sodium, used as the standard, demonstrated inhibition ranging from **16.98% at 20 µg/mL to 86.79% at 100 µg/mL**. Quercetin demonstrated inhibition ranging from **9.96% to 58.01%**, whereas Phytol showed inhibition ranging from **4.55% to 55.63%** over the tested concentration range.

The Quercetin–Phytol cream demonstrated inhibition ranging from **12.12% at 20 µg/mL to 68.40% at 100 µg/mL**. The formulation therefore demonstrated greater inhibition of protein denaturation at the higher concentrations compared with the individual Quercetin and Phytol treatments.

Table 3. Effect of test samples on protein denaturation

Treatment	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	IC_{50} (µg/mL)*
Diclofenac sodium	16.98%	25.32%	42.76%	59.11%	86.79%	70.89
Quercetin	9.96%	17.75%	31.82%	46.54%	58.01%	92.70
Phytol	4.55%	12.55%	25.11%	41.77%	55.63%	94.57
Quercetin + Phytol cream	12.12%	21.21%	41.56%	55.19%	68.40%	72.09

At **100 µg/mL**, the Quercetin–Phytol cream produced **68.40% inhibition**, compared with **58.01%** for Quercetin and **55.63%** for Phytol individually.

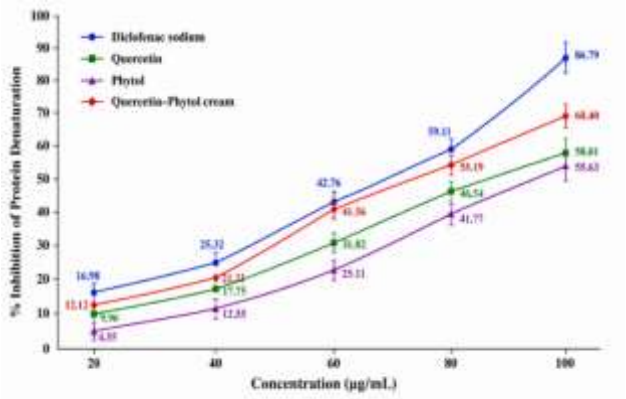


Figure 2. Concentration-dependent inhibition of protein denaturation by Diclofenac sodium, Quercetin, Phytol and Quercetin–Phytol cream.

3.5 HRBC Membrane Stabilization Assay

The HRBC membrane stabilization assay demonstrated a concentration-dependent increase in membrane stabilization for Aspirin, Quercetin, Phytol and Quercetin–Phytol cream.

The Quercetin–Phytol cream demonstrated membrane stabilization ranging from **13.01% at 20 µL/mL to 64.50% at 100 µL/mL**.

Aspirin, used as the standard, showed membrane stabilization ranging from **70.73% at 20 µL/mL to 93.50% at 100 µL/mL**. Quercetin demonstrated membrane stabilization ranging from **14.36% to 53.39%**, whereas Phytol showed an increase from **7.86% to 51.76%** over the tested concentration range.

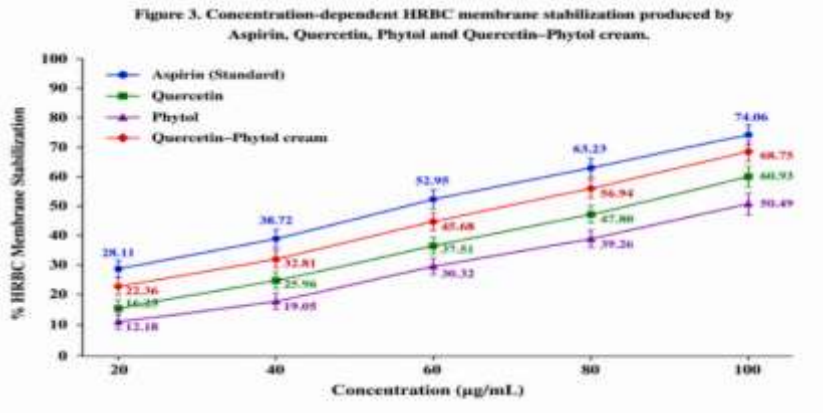
At the highest tested concentration, the Quercetin–Phytol cream demonstrated greater membrane stabilization than Quercetin and Phytol individually, although the standard Aspirin showed the highest activity.

Table 4. Effect of test samples on HRBC membrane stabilization

Treatment	20 µL/mL	40 µL/mL	60 µL/mL	80 µL/mL	100 µL/mL
Aspirin	70.73%	76.42%	87.80%	90.24%	93.50%

Quercetin	14.36%	26.56%	37.13%	48.24%	53.39%
Phytol	7.86%	15.18%	30.62%	40.38%	51.76%
Quercetin + Phytol cream	13.01%	19.51%	39.30%	50.14%	64.50%

The maximum membrane stabilization produced by the optimized Quercetin–Phytol cream was **64.50% at 100 µL/m**



3.6 Statistical Analysis

The experimental data obtained from the anti-inflammatory assays were subjected to statistical analysis using the replicate observations. Two-way analysis of variance (ANOVA) was used to assess the effects of **treatment and concentration**, as well as their **combined interaction**, on the observed results.

In the HRBC membrane stabilization assay, significant differences were observed for **treatment, concentration, and their interaction ($p < 0.05$)**. The treatment effect was highly significant (**$F = 1993.14$, $p = 1.44 \times 10^{-43}$**), while a significant effect was also observed for concentration (**$F = 656.74$, $p = 6.87 \times 10^{-36}$**). The interaction between treatment and concentration was also found to be statistically significant (**$F = 18.08$, $p = 1.67 \times 10^{-12}$**), indicating that the response varied with changes in both treatment and concentration.

Table 5. Two-way ANOVA of HRBC membrane stabilization assay

Source	SS	df	MS	F-value	p-value
Treatment	4.2753	3	1.4251	1993.14	1.44×10^{-43}
Concentration	1.8783	4	0.4696	656.74	6.87×10^{-36}
Treatment × Concentration	0.1551	12	0.0129	18.08	1.67×10^{-12}
Error	0.0286	40	0.000715	—	—
Total	6.3373	59	—	—	—

The statistically significant treatment effect indicates that the type of treatment significantly influenced HRBC membrane stabilization. The significant concentration effect demonstrates that increasing concentration significantly affected membrane stabilization. The significant interaction indicates that the effect of concentration was not identical across all treatments.

Overall, the results of the physicochemical evaluation, Franz diffusion study and in-vitro anti-inflammatory assays demonstrated that the optimized Quercetin–Phytol cream possessed satisfactory pharmaceutical characteristics and exhibited concentration-dependent biological activity in the selected in-vitro models.

4. DISCUSSION

4.1 Formulation Development and Optimization

The formulation development study was carried out to prepare a **Quercetin–Phytol cream** with suitable physical properties and good performance for topical application. A total of **seven preliminary trial formulations (F1–F7)** were prepared by varying the concentrations of selected excipients, while keeping the active ingredients as per the planned formulation design. The variation in excipient concentration was intended to optimize the consistency, homogeneity, spreadability and overall handling characteristics of the cream. Such systematic adjustment of excipients is an important aspect of semisolid formulation development because the composition of the cream base can influence its consistency, application properties and overall performance. [14,15]

Among the preliminary batches, **F5 was selected as the optimized formulation** because it showed comparatively better physical characteristics and was suitable for further evaluation. The selection of F5 allowed the cream base to be optimized without making unnecessary changes to the concentration of the active ingredients.

The final optimized batch was prepared on a 30-g scale and contained 0.30 g of Quercetin and approximately 100 mg of Phytol. The formulation also contained stearic acid, cetyl alcohol, liquid paraffin, Span-20, Tween-80, glycerin, propylene glycol, methyl paraben and purified water. The final formulation was designed as an oil-in-water cream suitable for topical administration. The selection of appropriate emulsifying, consistency-enhancing, emollient and humectant components is important for obtaining a physically acceptable topical semisolid preparation. [14,15]

This formulation approach was considered important because **Quercetin has well-known antioxidant and anti-inflammatory properties**, but its use in topical formulations can be limited by its **poor solubility in water and challenges associated with effective skin delivery**. [5,6]

Phytol is a naturally occurring diterpene alcohol that has also been investigated for various biological activities. [7] Thus, incorporation of Quercetin and Phytol into a common topical dosage form provided a rational approach for investigating their combined formulation performance.

4.2 Physicochemical Evaluation

The optimized formulation demonstrated satisfactory organoleptic characteristics, with a pale-yellow colour, pleasant rose odour, smooth texture and homogeneous appearance. These characteristics indicate acceptable physical uniformity and suggest that the formulation components were adequately incorporated into the cream base. Evaluation of appearance, homogeneity, consistency and related properties is routinely considered during the development of topical semisolid formulations. [14,15]

The pH of the optimized cream was found to be 5.24. The slightly acidic pH is appropriate for a topical formulation and is expected to provide better compatibility with the skin compared with highly acidic or alkaline preparations. Therefore, the observed pH supports the suitability of the formulation for topical application. [14,15]

The formulation exhibited a spreadability value of 7.69, indicating satisfactory ability of the cream to spread over the skin surface. Good spreadability is an important characteristic of topical preparations because it facilitates uniform application and distribution of the formulation over the affected area. [14,15]

The viscosity of the optimized cream was found to be 5760–5940 cP at 10 rpm, while a viscosity of approximately 9000 cP at 5 rpm was observed. The observed viscosity indicates that the formulation possessed adequate consistency for handling and topical application while retaining sufficient structure to remain at the site of application. The change in apparent viscosity with rotational speed is also consistent with the flow

behaviour expected from structured semisolid systems. [14,15]

The extrudability of the cream was found to be 90%, with approximately 9.0 g of cream being extruded from a 10-g sample under the specified test conditions. This indicates good ease of extrusion from the collapsible tube and supports convenient administration of the formulation. Extrudability is an important practical characteristic of creams intended for tube-based topical application. [14,15]

The drug content was found to be 97.5%, indicating satisfactory incorporation and relatively uniform distribution of Quercetin within the optimized cream. A drug content close to the theoretical value is desirable because it suggests that the formulation process did not result in substantial loss of the active ingredient.

Overall, the physicochemical evaluation showed that the optimized cream had **satisfactory appearance, homogeneity, pH, spreadability, viscosity, extrudability, and drug content**. The results indicated that the formulation had suitable physical properties and could therefore be taken forward for **further in-vitro evaluation**..

4.3 In-vitro Franz Diffusion

The Franz diffusion study was performed to investigate the diffusion behaviour of Quercetin from the optimized Quercetin–Phytol cream. The study employed a cellulose acetate membrane, phosphate-buffered saline as receptor medium and a temperature of 37 ± 1°C, with samples collected at 0, 1, 3, 6 and 24 h and analyzed at 370 nm. Franz diffusion-cell systems are widely used as in-vitro models for evaluating drug release and membrane diffusion behaviour from topical preparations. [16]

The cumulative diffusion demonstrated a progressive increase with increasing diffusion time. The mean cumulative diffusion increased from approximately 1.06% at 0 h to 2.70% at 1 h, 3.21% at 3 h, 5.20% at 6 h and 6.39% at 24 h. The gradual increase in diffusion over the study period indicates that Quercetin was continuously released from the cream matrix rather than being released immediately.

The relatively gradual diffusion pattern is desirable for a topical formulation because prolonged availability of the active ingredient at the application site may support sustained local exposure. The diffusion profile therefore indicates that the cream matrix was capable of controlling the release of Quercetin over the investigated period. However, the present experiment demonstrates in-vitro diffusion through the selected membrane, and this should not be interpreted directly as equivalent to drug permeation through human skin. [16]

Kinetic evaluation showed strong correlation with the first-order model ($R^2 = 0.9994$), while the Higuchi model also demonstrated a high correlation ($R^2 = 0.9018$). The reported Korsmeyer–Peppas model showed an R^2 value of 1.000.

The diffusion results therefore indicate a controlled and gradual release behaviour of Quercetin from the optimized cream. However, because the study was an in-vitro membrane diffusion study, the results should be interpreted as evidence of release/diffusion behaviour rather than direct evidence of clinical skin permeation or therapeutic efficacy.

4.4 Protein Denaturation Activity

The protein denaturation assay demonstrated a concentration-dependent increase in inhibition of protein denaturation for all tested samples. Protein-denaturation inhibition is commonly used as a preliminary in-vitro approach for screening anti-inflammatory activity. [17]

Diclofenac sodium, used as the standard, exhibited the highest activity, with inhibition increasing from 16.98% at 20 µg/mL to 86.79% at 100 µg/mL. Its reported IC_{50} was 70.89 µg/mL.

Quercetin demonstrated inhibition ranging from 9.96% to 58.01%, with a reported IC_{50} of 92.70 µg/mL. The observed activity is consistent with the reported anti-inflammatory potential of Quercetin. [5,6]

Phytol showed inhibition ranging from 4.55% to 55.63%, with a reported IC_{50} of 94.57 µg/mL. The observed concentration-dependent response provides preliminary support for the biological activity of Phytol under the experimental conditions. [7]

The Quercetin–Phytol cream showed a stronger concentration-dependent response, with inhibition increasing from 12.12% at 20 µg/mL to 68.40% at 100 µg/mL. The reported IC_{50} of the formulation was 72.09 µg/mL, which was considerably lower than the IC_{50} values of Quercetin and Phytol individually and relatively close to that of Diclofenac sodium.

The comparatively greater activity of the combination formulation may be associated with the contribution of

both active components. Quercetin provides a direct anti-inflammatory and antioxidant contribution, while Phytol has also been investigated for biological and anti-inflammatory activities. [5–8] The improved response of the combination compared with the individual components supports the potential benefit of incorporating both components into a single formulation.

However, the present results demonstrate enhanced activity of the combination, rather than definitively proving pharmacological synergy. Formal synergy assessment would require a dedicated combination-index or equivalent experimental approach. Therefore, the present findings are more appropriately interpreted as evidence of improved anti-inflammatory activity of the combined formulation under the tested conditions.

4.5 HRBC Membrane Stabilization

The HRBC membrane stabilization assay demonstrated a concentration-dependent increase in membrane stabilization for all tested samples. The HRBC membrane stabilization model is used as a preliminary in-vitro approach for evaluating membrane-protective and anti-inflammatory activity. [18]

Aspirin, used as the standard, showed membrane stabilization increasing from 70.73% at 20 µL/mL to 93.50% at 100 µL/mL. Aspirin therefore demonstrated the highest activity throughout the tested concentration range.

Quercetin showed an increase in membrane stabilization from 14.36% at 20 µL/mL to 53.39% at 100 µL/mL, whereas Phytol demonstrated an increase from 7.86% to 51.76%. The observed responses are consistent with the biological activity reported for these naturally occurring compounds. [5–8]

The Quercetin–Phytol cream demonstrated membrane stabilization ranging from 13.01% at 20 µL/mL to 64.50% at 100 µL/mL. At the highest concentration tested, the formulation showed greater membrane stabilization than Quercetin and Phytol individually, although its activity remained lower than that of Aspirin.

The greater membrane-stabilizing activity observed with the combination formulation suggests that incorporation of Quercetin and Phytol into the cream produced a stronger response than either component alone at the higher concentrations. Since **erythrocyte membrane stabilization is considered an in-vitro indicator of protection against membrane damage during inflammatory processes**, the observed activity provides supportive evidence for the **potential anti-inflammatory effect of the developed formulation**. [18]

Nevertheless, the HRBC assay is an in-vitro screening model, and the observed membrane-stabilizing activity should not be interpreted as direct evidence of clinical anti-inflammatory efficacy.

4.6 Overall Interpretation

The overall findings indicate that the optimized Quercetin–Phytol cream possessed satisfactory pharmaceutical characteristics and demonstrated measurable in-vitro anti-inflammatory activity.

The formulation development study resulted in selection of F5 as the optimized preliminary formulation, followed by preparation of the final 30-g cream containing Quercetin and approximately 100 mg of Phytol. The optimized formulation showed satisfactory physical characteristics, including homogeneous appearance, acceptable pH, satisfactory spreadability, appropriate viscosity and good extrudability. These characteristics are important considerations in the development of topical semisolid dosage forms. [14,15]

The 97.5% drug content further indicated satisfactory incorporation and distribution of Quercetin within the cream. The Franz diffusion study demonstrated a gradual increase in Quercetin diffusion over 24 h, supporting the ability of the formulation to provide prolonged release of the active ingredient under the experimental conditions. [16]

The two in-vitro anti-inflammatory assays provided complementary evidence of biological activity. The protein denaturation assay showed a concentration-dependent increase in inhibition, with the Quercetin–Phytol cream producing 68.40% inhibition at 100 µg/mL and a reported IC_{50} of 72.09 µg/mL. Similarly, the HRBC membrane stabilization assay demonstrated 64.50% membrane stabilization at 100 µL/mL, exceeding the responses observed with Quercetin and Phytol individually but remaining below the standard Aspirin. The use of these two models provides complementary preliminary evidence of anti-inflammatory activity. [17,18]

Taken together, these findings suggest that the optimized Quercetin–Phytol cream has promising pharmaceutical and in-vitro anti-inflammatory characteristics. The

combination of satisfactory formulation properties, gradual Quercetin diffusion and concentration-dependent activity in the protein-denaturation and HRBC membrane-stabilization assays supports further investigation of the formulation.

However, these findings are limited to in-vitro evaluation and should not be interpreted as proof of clinical efficacy or superiority over established treatments. Further studies, such as **detailed skin permeation studies, stability testing, and suitable biological or clinical investigations**, are needed to confirm the **therapeutic potential and effectiveness of the developed formulation**

5. Conclusion

The present study successfully developed and evaluated a **Quercetin–Phytol topical cream** for its potential anti-inflammatory application. Out of the seven preliminary formulations, **F5 was selected as the optimized formulation** based on its satisfactory physical and formulation characteristics. The selected formulation was then prepared on a **30-g scale for further evaluation**.

The optimized cream demonstrated satisfactory physicochemical properties, including acceptable appearance and homogeneity, a skin-compatible pH, satisfactory spreadability, suitable viscosity and good extrudability. The formulation showed a **drug content of 97.5%**, indicating satisfactory incorporation and distribution of Quercetin within the cream.

The **in-vitro Franz diffusion study** showed that Quercetin gradually diffused from the cream over the **24-hour period**, indicating a sustained release of the drug from the formulation. The **protein denaturation and HRBC membrane stabilization studies** also showed an increase in anti-inflammatory activity with increasing concentrations of the optimized **Quercetin–Phytol cream**. These findings suggest that the developed formulation has promising **in-vitro anti-inflammatory potential**. The formulation showed greater activity than Quercetin and Phytol individually at the higher tested concentrations, although the respective standard drugs exhibited higher activity.

Overall, the findings indicate that the optimised **Quercetin–Phytol cream possesses satisfactory pharmaceutical characteristics and promising in-vitro anti-inflammatory potential**. The formulation may therefore serve as a suitable candidate for further investigation as a topical anti-inflammatory preparation. However, the present findings are limited to formulation evaluation, in-vitro diffusion and in-vitro anti-inflammatory assays; further studies are required to establish its stability, skin permeation, safety and therapeutic efficacy.

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