

Factorial Design- Guided Development and Optimization of Quercetin- Enriched *Thuja orientalis* Emulgel for Collagenase Inhibition

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Abstract

Objective: To formulate and optimize an emulgel incorporating the quercetin enriched fraction of *Thuja orientalis* for topical application and to evaluate its physicochemical properties, in vitro release characteristics, skin irritation potential, and anti-collagenase activity for potential anti-aging applications.

Methods: Dried leaves of *Thuja orientalis* were extracted sequentially with petroleum ether, chloroform, and ethanol using a Soxhlet apparatus. The crude ethanolic extract was subsequently partitioned with ethyl acetate. A 3² factorial design was applied to optimize leaf emulgel formulations (F1–F9), selecting Carbopol 940 (1–2% w/w) and an emulsifying blend of Tween 80 and Span 80 (0.9–1.5% w/w) as independent variables. The evaluated responses included viscosity, spreadability, and cumulative drug release over 8 hours. The optimized emulgel was characterized using Fourier-transform infrared spectroscopy (FTIR), in vitro release kinetics, primary skin irritation testing in Wistar rats, collagenase inhibition assays, and a 6-month accelerated stability study.

Results: Across all formulations, viscosity ranged between 3256 and 11,920 cP, spreadability from 12.35 to 25.35 g·cm/s, and drug release from 94.57% to 98.46%. Statistical analysis revealed Carbopol 940 as the primary determinant; increasing its concentration significantly elevated viscosity while reducing spreadability and drug release ($p \leq 0.0001$). The emulsifier mixture influenced viscosity ($p = 0.0059$) without markedly affecting spreadability or release. The optimized batch (1.43% Carbopol 940 and 1.33% emulsifier) yielded a viscosity of 8272 ± 253 cP, spreadability of 18.2 ± 0.74 g·cm/s, and drug release of $97.49 \pm 0.39\%$, aligning within 8% of predicted values. Release profiles best fitted the Higuchi diffusion model ($R^2 = 0.9928$). FTIR spectra confirmed the absence of drug-excipient interactions, and in vivo safety studies showed no edema or erythema over 14 days. The formulation demonstrated dose-dependent collagenase inhibition ($IC_{50} = 75.24$ µg/mL) comparable to EDTA ($IC_{50} = 50.87$ µg/mL). Accelerated testing (40 °C / 75% RH) confirmed stability for both leaf and bark emulgels over 6 months.

Conclusion: A stable, non-irritant, and readily spreadable emulgel of *T. orientalis* quercetin enriched fraction was successfully developed. The factorial approach clarified the distinct roles of the gelling agent and emulsifying pair in controlling formulation mechanics. Promising anti-collagenase activity supports its potential application as a topical anti-aging formulation.

Keywords: *Thuja orientalis*; *Platyclusus orientalis* ; emulgel; Carbopol 940; 3² factorial design; collagenase inhibition; anti-aging; topical delivery

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Introduction

Skin ageing is a natural process that cannot be avoided, and it slowly changes the strength, structure and elasticity of the skin [1]. In the extracellular matrix, collagen and elastin act as the main supporting fibres, and protease enzymes such as collagenase and elastase break them down; exposure to ultraviolet light adds to the damage by raising the level of reactive oxygen species [2]. Since loss of collagen is one of the clearest signs of ageing, there is a steady demand for compounds that can hold back collagenase [2]. Collagenase inhibition, along with antioxidant, anti-elastase and anti-tyrosinase tests, is also among the in vitro

assays most often used to judge the anti-ageing potential of a compound or a cosmetic product [1]. Plants are a rich and widely studied source of natural compounds for this purpose. One review of in vitro and in vivo studies listed 119 medicinal plants and 50 isolated compounds with anti-ageing potential [3]. Plant constituents are reported to help the skin by scavenging free radicals and by holding back enzymes such as hyaluronidase, collagenase and elastase [4], and a systematic review of plant-based cosmetic ingredients described the same antioxidant, anti-inflammatory and enzyme-inhibiting actions [5]. Screening work points the same way. Of the plants tested in one study, sixteen lowered

collagenase activity, and white tea was the most active [6]. In a screen of 140 medicinal plants, 71 extracts inhibited collagenase, and the effect increased with the phenolic content of the extract [7]. Collagenase inhibition has also been reported for leaf extracts of Thai herbs [8] and for red pitaya peel [9], and plant extracts have even been used to prepare nanoparticles that show anti-ageing activity [10]. Many of these reports link the activity to phenolic compounds, and especially to flavonoids. In an NMR-based study of six herbs, the most active one, *Persicaria minus*, contained quercetin and several related flavonoids [11], and Greek propolis samples rich in flavonoids showed the strongest collagenase inhibition [12].

Thuja orientalis L. (also known as *Platycladus orientalis*) belongs to the family Cupressaceae and is an evergreen, monoecious tree or shrub [13,14]. The related species *Thuja occidentalis* is widely used in homeopathy and herbal medicine [15]. *T. orientalis* has traditionally been used for bronchial catarrh, cystitis, psoriasis, rheumatism and skin infections [13,14], and in Northeast Asia it is used for inflammatory conditions such as dermatitis [16]. Reviews describe antioxidant, anticancer, anti-inflammatory, antibacterial, antifungal, antiviral and hair growth-promoting effects of different parts of the plant [13,14].

The chemistry of *T. orientalis* has been studied in several plant parts. Its essential oil is rich in monoterpenes, and α -pinene is the main component of the oil from the fruits [17] as well as from the needles and cones [18]. In Thuja oils, thujone, fenchone and camphor are also prominent, and the plants contain flavonoids such as rutin, quercitrin, quercetin and amentoflavone [19]. Nine flavonoids isolated from the fruits were tested for activity: several scavenged free radicals, and three biflavonoids inhibited human neutrophil elastase [20]. Diterpenoids have been isolated from the leaves as well. A labdane-type diterpene from the ethyl acetate fraction of a methanolic leaf and stem extract suppressed inflammatory responses in macrophage cells [16]. Other leaf diterpenoids were screened for cytotoxicity against cancer cell lines [21], and some were found to inhibit PTP1B and α -glucosidase [22].

Crude extracts and fractions of the plant have also shown useful biological effects. A Soxhlet extract of the leaves showed antioxidant and antibacterial activity [23]. Extracts in different solvents reduced hydrogen peroxide-induced haemolysis of red blood cells in a dose-dependent way, and the water extract had a stronger antioxidant effect than the ethyl acetate, ethanol and methanol extracts [24]. Extracts of *T. orientalis*, also studied under the name *Platycladus orientalis*, were active against Gram-positive bacteria [25], and a leaf extract was used to prepare iron oxide nanoparticles with antibacterial and antifungal activity [26]. Compounds from this

plant have been reported to inhibit elastase [17,20], but among the reports reviewed for this work, none described collagenase inhibition by a finished topical product of *T. orientalis*.

Taking a plant extract to the skin is not simple. Extracts are mixtures, many of their active constituents are lipophilic, and these do not disperse well in a plain water-based gel. Problems of stability and skin delivery have also been noted for plant-derived anti-ageing ingredients, and carrier systems are being sought to overcome them [5]. Emulgels offer one practical answer. An emulgel joins an emulsion with a gel base, so that a lipophilic material can be carried in the oil phase while the gel gives a smooth product that is easy to spread [27]. Emulgels have been made from several herbal materials, for example *Ocimum basilicum* for wound healing [28], *Markhamia tomentosa* leaf extract tested against skin isolates [29], and a polyherbal mixture for rheumatoid arthritis [30]. The properties of an emulgel depend on its composition, mainly the gelling agent and the emulsifier, so the composition must be chosen with care, and the optimization of emulgel composition has been reported earlier, for example for a diclofenac emulgel [31].

In the present work, dried leaves of *T. orientalis* were extracted in succession with solvents of rising polarity, and the ethanolic extract was partitioned with ethyl acetate to obtain the fraction used for formulation. An ethyl acetate fraction of this plant has been linked with anti-inflammatory activity before [16], and the way an extract is prepared can change both its yield and its activity [32]. The aim was to develop and optimize an emulgel of the quercetin enriched fraction using a 3^2 factorial design, with Carbopol 940 and the Tween 80:Span 80 emulsifier mixture as the two factors and viscosity, spreadability and drug release at 8 h as the responses. The optimized emulgel was then examined by FTIR, release kinetics, skin irritation in Wistar rats and collagenase inhibition, and the stability of the leaf and bark emulgels was followed for 6 months under accelerated conditions.

Materials and Methods

Plant material

Leaves of *Thuja orientalis* [13,14] were collected from naturally occurring specimens in Nashik, Maharashtra, India. The collected plant material was botanically identified and authenticated by D.L. Shirodkar, Botanist, Western Regional Circle, Botanical Survey of India, Pune, through comparative morphological assessment against authenticated herbarium specimens. A representative voucher specimen (Voucher No. SDMP0-01; Reference No. BSI/ WRC /Iden. Cert. /2022/0212220021253) has been deposited at the institutional herbarium for scientific reference and traceability. The leaf materials were separated, washed shade-dried at ambient temperature. The dried material was then crushed in a grinder and

passed through sieve No.40 to obtain an even particle size distribution. Carbopol 940, Tween 80, Span 80, sesame oil, methylparaben, propylene glycol, triethanolamine, EDTA, collagenase and the substrate N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) were of laboratory or analytical grade.

Methods

Extraction and Fractionation

Separately weighed 100 g quantities of dried leaves powdered of *Thuja orientalis* were successively extracted in a Soxhlet apparatus with petroleum ether, chloroform, and alcohol [23,32]. Each extraction was continued until exhaustion, and completion was confirmed by TLC. The respective solvents were removed to obtain the dried extracts. Percentage yield of the alcoholic extract was calculated & extract yield = 13.2% (w/w). The concentrated alcoholic extract was dissolved in distilled water and subjected to liquid-liquid extraction with ethyl acetate using a separating funnel [16]. The ethyl acetate layer was separated, collected, concentrated, and dried to obtain the ethyl acetate-soluble fraction. Percentage extract yield of the ethyl acetate soluble fraction from the crude extract was calculated on a dry weight basis, Leaf extract yield = 4.5% (w/w). Subsequent to further separation of these fractions using column chromatography, each collected fraction was analyzed with TLC. The fractions that showed chromatographic profiles corresponding quercetin were selected for inclusion in the emulgel formulation. Quercetin Content in the Ethyl Acetate-Soluble Fraction by gravimetric determination is, leaf fraction = 1.8%. Preliminary phytochemical screening confirmed the presence of flavonoids and phenolic compounds in both leaf extracts, supporting earlier reports on *Thuja orientalis* [13,14,19].

Evaluation: appearance, FTIR, pH, viscosity, spreadability in vitro release and kinetics, collagenase inhibition, accelerated stability (40 °C/75% RH, 6 months)

Figure 1: Workflow of extraction, fractionation, emulgel preparation and evaluation.

Preparation of the Emulgels

The gel phase was prepared by dispersing Carbopol 940 in purified water under continuous stirring until a homogeneous gel matrix was formed. Separately, the oil phase was prepared by dissolving Span 80 in sesame oil. For the aqueous phase, Tween 80 was dissolved in purified water, followed by the addition of methylparaben (previously dissolved in propylene glycol) and the quercetin enriched fraction (dissolved in ethanol). Both the oil and aqueous phases were heated independently to 65–70 °C under constant agitation. The oil phase was subsequently introduced dropwise into the aqueous phase under continuous stirring to yield a primary emulsion. The pH was adjusted to 6.1–7.3 using

triethanolamine, after which the emulsion was uniformly blended with the gel phase to obtain the final emulgel formulation [27–29]. The optimized leaf-derived emulgel was designated as TOLEF. A control placebo emulgel lacking the active extract fraction was prepared following the same procedure.

Experimental Design and Optimization

A 3² full factorial design was applied with Design-Expert software [31]. The two independent variables were the concentration of Carbopol 940 (X₁) and the concentration of the emulsifier mixture (X₂), each at three levels (Table 1). In the emulsifier mixture, Tween 80 was fixed at 0.3% w/w while Span 80 was varied (0.6, 0.9 and 1.2% w/w), which gave Tween 80: Span 80 ratios of 1:2, 1:3 and 1:4. The fraction content was kept at 1% w/w in all batches. The nine batches (F1–F9) are listed in Table 2. Viscosity (Y₁), spreadability (Y₂) and drug release at 8 h (Y₃) were the dependent variables. The data were analysed by analysis of variance (ANOVA), and terms with p < 0.05 were taken as significant. Contour and three-dimensional response-surface plots were drawn, and numerical optimization was used to select the best factor combination. The optimized emulgel was then prepared and tested, and the observed responses were compared with the predicted ones.

Table 1: Variables and levels of the 3² factorial design

Independ ent variable	Low (–1)	Mediu m (0)	High (+1)	Depende nt variable
X ₁ : Carbopol 940 (% w/w)	1	1.5	2	Y ₁ : Viscosity
X ₂ : Emulsifie r, Tween 80:Span 80 (% w/w)	0.3:0 .6 (1:2)	0.3:0.9 (1:3)	0.3:1 .2 (1:4)	Y ₂ : Spreadabi lity Y ₃ : Drug release at 8 h

Table 2: Composition (% w/w) of the emulgel batches F1–F9

Ingredient	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
<i>T. orientalis</i> fraction	1	1	1	1	1	1	1	1	1
Carbopol 940	1	1	1	1.5	1.5	1.5	2	2	2
Emulsifier (Tween 80 + Span 80)	0.9	1.2	1.5	0.9	1.2	1.5	0.9	1.2	1.5
Sesame oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methyl paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propylene glycol	5	5	5	5	5	5	5	5	5
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Characterization of the Emulgels Organoleptic and Physical Evaluation

The prepared emulgels were visually inspected for color, homogeneity, consistency, and any evidence of phase separation [27,28].

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the active fraction, the leaf-derived emulgel, and the placebo emulgel were acquired in the wave number range of 4000 to 650 cm⁻¹ using potassium bromide (KBr) pellet dispersions under standardized experimental conditions to assess drug–excipient compatibility.

pH Determination

1 g of emulgel was dissolved in 25 mL of distilled water. The pH of each formulation was measured at room temperature (25 ± 2 °C) using a calibrated digital pH meter [27,29].

Viscosity

Rheological properties were evaluated using a Brookfield viscometer equipped with spindle no. 64 at a rotational speed of 100 rpm and a controlled temperature of 25 °C [27,31].

Spreadability

Spreadability was quantified via the parallel-plate (slip-and-drag) technique [28,31]. Briefly, 1 g of emulgel was placed between two glass plates, and

the time (*T*, in seconds) required for a standard weight (*M* = 20 g) to pull the upper slide across a fixed distance (*L* = 7 cm) was recorded. Spreadability (*S*) was calculated using the equation: $S = (M \times L) / T$

Higher *S* values indicate spreadability and ease of topical application.

In Vitro Drug Release and Kinetic Modeling

Permeation studies were carried out using a Franz diffusion cell [27,28,30] featuring a 25 mL receptor compartment. Goat skin, pre-conditioned in phosphate buffer for 24 h, was mounted between the donor and receptor chambers, and 1 g of emulgel was uniformly applied to the skin surface. Phosphate-buffered solution (pH 6.8) served as the receptor fluid. Samples were periodically withdrawn over an 8-hour period and analyzed spectrophotometrically. The cumulative percentage of drug released at 8 h served as the target response variable. To elucidate the release mechanism of the optimized formulation, the release data were fitted into zero-order, first-order, Higuchi, and Korsmeyer–Peppas mathematical models, comparing goodness-of-fit via their respective determination coefficients (*R*²).

Skin Irritation Study

Healthy Wistar albino rats of either sex (weighing 180–250 g) were divided randomly into three groups of six animals each: placebo emulgel, leaf-derived emulgel (TOLEF). The animals were housed in standard laboratory cages maintained under controlled environmental conditions with a 12-hour light/dark cycle. They were provided with a standard rodent pellet diet and water. Prior to experimentation, all rats were acclimatized to the laboratory environment for one week. The study was approved by the Institutional Animal Ethics Committee (approval no. SIPS/CCSEA/IAEC/2026-27/02-1858). About 24 h before the test, hair on the back was clipped carefully without hurting the skin, and only animals with healthy, intact skin were used. The emulgel was spread evenly on the prepared area, which was covered with sterile gauze and held with a non-irritating dressing for 4 h under semi-occlusive conditions. After 4 h the dressing was removed and any remaining emulgel was wiped off gently. The sites were examined for erythema, oedema and other visible reactions 1 h, 24 h, 48 h and 72 h after removal of the patch, and follow-up was continued to day 14 to see whether any reaction persisted or reversed. General condition, behaviour, signs of toxicity and mortality were also recorded. The procedure follows the general principles of acute dermal irritation testing.

Collagenase Inhibition Assay

Collagenase inhibition was measured with a modified spectrophotometric method that uses the synthetic substrate FALGPA [2,6,9]. Collagenase was prepared in 50 mM Tricine buffer (pH 7.5)

containing 10 mM calcium chloride. The reaction mixture contained 100 μ L of test sample at different concentrations (20, 40, 60, 80 and 100 μ g/mL), 50 μ L of enzyme solution and 50 μ L of buffer. It was pre-incubated at 37 $^{\circ}$ C for 15 min, and the reaction was started by adding 100 μ L of FALGPA. Absorbance was read immediately at 335 nm in a UV-visible spectrophotometer. EDTA was used as the standard inhibitor, and all measurements were made in triplicate. Percentage inhibition was calculated as $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, and the IC_{50} value was obtained from the concentration-response data.

Stability Study

Stability was tested according to the ICH guideline. The optimized emulgels were filled in aluminium containers covered with polyethylene and kept at 40 ± 2 $^{\circ}$ C and $75 \pm 5\%$ relative humidity (accelerated condition) for 6 months. Appearance, pH, viscosity, spreadability and drug release at 8 h were checked at the start and after 3 and 6 months.

Statistical Analysis

Experimental data are expressed as mean $\pm$ standard deviation ($n = 3$ for formulation characterization assays and anti-collagenase evaluations; $n = 6$ per group for in vivo skin irritation studies). Analysis of variance (ANOVA), response surface methodology, model fitting, and multi-response optimization were performed using Design-Expert software. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Physical Appearance

All nine batches were white, smooth and homogeneous, with excellent consistency and no sign of phase separation. The formulations were thus visually uniform, and differences among them were examined through the measured responses described below.

FTIR and Compatibility

The spectrum of the leaf fraction (Figure 2a) showed a broad band at 3385.6 and 3364.6 cm^{-1} , which is typical of O-H stretching of phenolic and alcoholic groups, and a band at 1636.8 cm^{-1} that falls in the region of C=O and C=C stretching of conjugated and aromatic systems [14]. Both are expected for a flavonoid-rich fraction. A few weak features between 2200 and 2000 cm^{-1} were at the level of baseline noise and were not assigned. The leaf emulgel and the placebo emulgel (Figure 2b,c) gave very similar spectra. Both showed the C-H stretching bands of the oil and emulsifier near 2923 and 2853 cm^{-1} , a band for the ester C=O of sesame oil triglycerides at about 1741 cm^{-1} , C-H bending bands near 1460 and 1376 cm^{-1} and a group of C-O stretching bands between 1240 and 1040 cm^{-1} (Table 3).

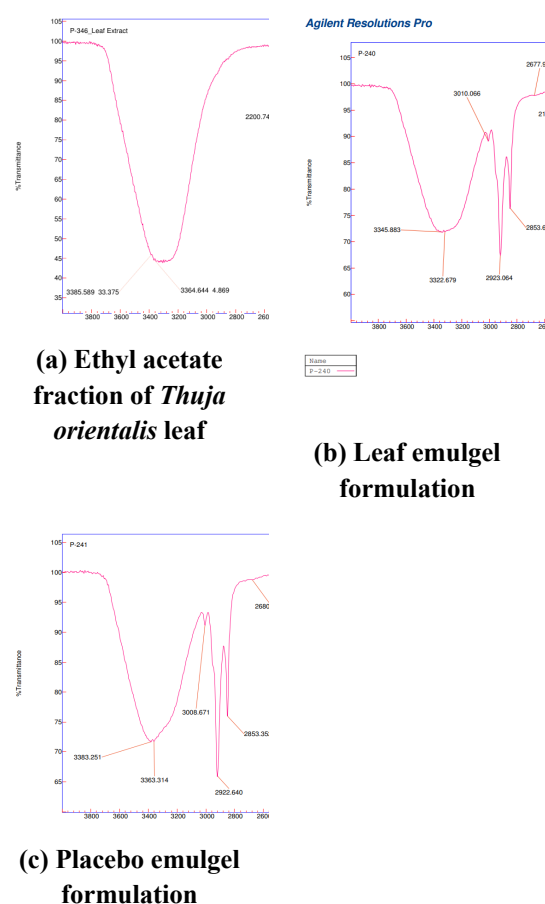


Figure 2: FTIR spectra of (a) the ethyl acetate fraction of *T. orientalis* leaf, (b) the leaf emulgel and (c) the placebo emulgel.

Table 3: Main FTIR bands (cm^{-1}) of the leaf fraction, the leaf emulgel and the placebo emulgel, with the usual group assignments

Assignment	Leaf fraction	Leaf emulgel	Placebo emulgel
O-H stretching	3385.6, 3364.6	3345.9, 3322.7	3383.3, 3363.3
=C-H stretching	—	3010.1	3008.7
C-H stretching (CH_2 , CH_3)	—	2923.1, 2853.6	2922.6, 2853.4
Ester C=O stretching	—	1741.4	1742.2
C=O / C=C stretching (conjugated, aromatic); bound water	1636.8	1650.6	1647.7
C-H bending	—	1459.6, 1376.1	1460.1, 1376.0, 1352.1
C-O stretching	—	1238.1, 1162.7, 1095.4, 1044.2	1238.2, 1160.9, 1094.1
Out-of-plane / CH_2 rocking region	660.9	914.7, 719.5	950.5, 719.7

The Fourier-transform infrared (FTIR) spectral data for the *Thuja orientalis* leaf fraction, leaf emulgel, and placebo emulgel are summarized in Table 3. Broad absorption bands in the 3322.7 to 3385.6 cm^{-1} region across all samples correspond to O-H stretching vibrations, indicating the presence of hydroxyl groups and hydrogen bonding. Distinct peaks observed in both the leaf emulgel and placebo emulgel at 3010.1 cm^{-1} (=C-H stretching), 2922.6 to 2923.1 cm^{-1} and 2853.4 to 2853.6 cm^{-1} (C-H stretching of CH_2 and CH_3 groups), and 1741.4 to 1742.2 cm^{-1} (ester C=O stretching) are attributed to the lipid/oil phase components present in the formulation base.

The peak at 1636.8 cm^{-1} in the leaf fraction, assigned to conjugated aromatic C=O/C=C stretching, shifted slightly to 1650.6 cm^{-1} in the formulation due to

non-covalent interactions within the emulgel matrix. Characteristic C–H bending vibrations (1376.0 to 1460.1 cm^{-1}) and C–O stretching bands (1044.2 to 1238.2 cm^{-1}) were consistently retained in the emulgel formulations. Crucially, the functional group absorption bands of the leaf emulgel closely mirrored those of the placebo emulgel and active fraction without the emergence of any new peak positions, confirming chemical compatibility and the absence of unfavorable interactions between the *T. orientalis* fraction and formulation excipients. The FTIR spectra of the placebo and drug-loaded emulgels exhibited similar absorption bands, which might be attributed to the predominant contribution of the excipients. The characteristic peaks of the quercetin-enriched fraction may be masked by overlapping excipient bands and its dispersion within the polymeric matrix.

pH, Viscosity and Spreadability of the Batches

The responses of the nine batches are collected in Table 4 and shown in Figure 3. The pH of all batches lay between 6.2 and 6.6. The pH was not affected in any regular way by the factor levels.

Table 4: pH, viscosity, spreadability and drug release at 8 h of emulgel batches F1–F9 (mean $\pm$ SD)

Batch	Carbopol (%)	Emulsifier (%)	pH	Viscosity (cP)	Spreadability (g·cm/s)	Release at 8 h (%)
F1	1	0.9	6.3 $\pm$ 0.17	3256 $\pm$ 20	25.35 $\pm$ 0.37	98.43 $\pm$ 0.52
F2	1	1.2	6.4 $\pm$ 0.10	5016 $\pm$ 26	22.07 $\pm$ 1.09	98.26 $\pm$ 0.10
F3	1	1.5	6.6 $\pm$ 0.10	5930 $\pm$ 20	21.23 $\pm$ 1.18	98.46 $\pm$ 0.21
F4	1.5	0.9	6.6 $\pm$ 0.10	8345 $\pm$ 20	16.96 $\pm$ 0.89	96.65 $\pm$ 0.60
F5	1.5	1.2	6.2 $\pm$ 0.06	9025 $\pm$ 20	15.56 $\pm$ 0.36	96.89 $\pm$ 0.43
F6	1.5	1.5	6.3 $\pm$ 0.06	9520 $\pm$ 20	16.42 $\pm$ 0.26	96.18 $\pm$ 0.51
F7	2	0.9	6.6 $\pm$ 0.10	10900 $\pm$ 20	14.15 $\pm$ 0.33	94.57 $\pm$ 0.77
F8	2	1.2	6.5 $\pm$ 0.12	11340 $\pm$ 20	12.89 $\pm$ 0.36	94.89 $\pm$ 0.64
F9	2	1.5	6.3 $\pm$ 0.06	11920 $\pm$ 20	12.35 $\pm$ 0.26	94.76 $\pm$ 0.44

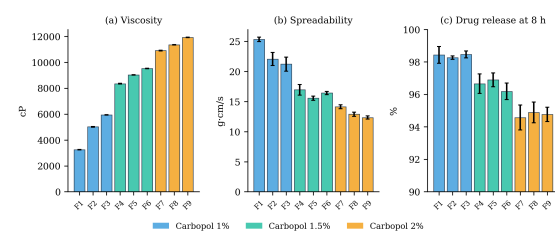


Figure 3: Effect of the formulation on (a) viscosity, (b) spreadability and (c) drug release at 8 h. Bars show mean $\pm$ SD; colours indicate the Carbopol 940 level.

Viscosity ranged from 3256 cP (F1) to 11,920 cP (F9). The average viscosity rose from about 4730 cP at 1% Carbopol to about 8960 cP at 1.5% and about 11,390 cP at 2%. At each Carbopol level, a higher emulsifier content also gave a more viscous product (for example 3256, 5016 and 5930 cP for F1–F3). Carbopol builds a swollen polymer network once it is neutralized, and more polymer gives a denser network and a thicker gel [27,31]. A larger amount of emulsifier probably adds to the structure by forming a stronger interfacial film around the oil

droplets, which would explain the smaller but regular increase.

Spreadability moved in the opposite direction to viscosity. It fell from 25.35 g·cm/s (F1) to 12.35 g·cm/s (F9), with mean values of about 22.9, 16.3 and 13.1 g·cm/s at 1, 1.5 and 2% Carbopol. This is the expected behaviour, since a thicker emulgel resists flow and needs more time to spread. An emulgel that is too thin runs off the skin, while one that is too thick is hard to apply, so a middle value is preferred [27].

Drug Release

The percentage of fraction released at 8 h ranged from 94.57% (F7) to 98.46% (F3) (Table 4, Figure 3c). The batches with 1% Carbopol released about 98.4% on average, those with 1.5% released about 96.6% and those with 2% released about 94.7%. A higher polymer level makes the gel network denser and more viscous, which slows the movement of the active constituents toward the membrane. A fall in release with a rise in gelling agent has been reported earlier for emulgels [27,31]. The emulsifier level had almost no effect on release. Even the slowest batch released more than 94% within 8 h, so all batches released the fraction readily.

Statistical Analysis of the Design

The ANOVA summary is given in Table 5. The model for viscosity was significant ($F = 181.58$, $p = 0.0006$) and contained a significant curvature term for Carbopol (A^2 , $p = 0.0204$). Carbopol had the largest effect ($F = 829.05$, $p < 0.0001$), the emulsifier effect was smaller but significant ($p = 0.0059$), and the interaction ($p = 0.0613$) and the emulsifier curvature ($p = 0.4994$) were not significant. The models for spreadability ($F = 40.95$, $p = 0.0003$) and for drug release ($F = 183.44$, $p < 0.0001$) were both linear. In both, Carbopol was the only significant factor ($p = 0.0001$ and $p < 0.0001$), and the emulsifier was not ($p = 0.0989$ and $p = 0.6767$). From the sums of squares in Table 5, the coefficient of determination (R^2) was 0.9967 for viscosity, 0.9318 for spreadability and 0.9837 for drug release, and the adjusted R^2 values were 0.9912, 0.9090 and 0.9785, so the models explain the data well.

Table 5: Summary of ANOVA for the 3² factorial design

Response / source	Sum of squares	df	Mean square	F-value	p-value
Viscosity (cP) — quadratic model; $R^2 = 0.9967$, adjusted $R^2 = 0.9912$					
Model	7.260×10^7	5	1.452×10^7	181.58	0.0006 (significant)
A: Carbopol	6.629×10^7	1	6.629×10^7	829.05	< 0.0001
B: Emulsifier	3.951×10^6	1	3.951×10^6	49.41	0.0059
AB	6.839×10^5	1	6.839×10^5	8.55	0.0613
A ²	1.622×10^6	1	1.622×10^6	20.29	0.0204
B ²	4.692×10^4	1	4.692×10^4	0.5868	0.4994
Residual	2.399×10^5	3	7.996×10^4		
Total	7.284×10^7	8			
Spreadability (g·cm/s) — linear model; $R^2 = 0.9318$, adjusted $R^2 = 0.9090$					
Model	149.65	2	74.82	40.95	0.0003 (significant)
A: Carbopol	142.69	1	142.69	78.10	0.0001
B: Emulsifier	6.96	1	6.96	3.81	0.0989
Residual	10.96	6	1.83		
Total	160.61	8			
Drug release (%) — linear model; $R^2 = 0.9837$, adjusted $R^2 = 0.9785$					
Model	19.92	2	9.96	183.44	< 0.0001 (significant)
A: Carbopol	19.91	1	19.91	366.69	< 0.0001
B: Emulsifier	0.0104	1	0.0104	0.1918	0.6767
Residual	0.3258	6	0.0543		
Total	20.25	8			

A, Carbopol 940; B, emulsifier mixture; df, degrees of freedom. R^2 and adjusted R^2 were calculated from the sums of squares in the table. Terms with $p < 0.05$ are significant.

-In coded units (-1 to +1 for each factor, with A for Carbopol and B for emulsifier), the fitted equations were:

$$\text{Viscosity} = -19671.2 + 20764A + 10924.4B - 2756.67AB - 3602.67A^2 - 1701.85B^2$$

$$\text{Spreadability} = 36.3789 - 9.75333A - 3.58889B$$

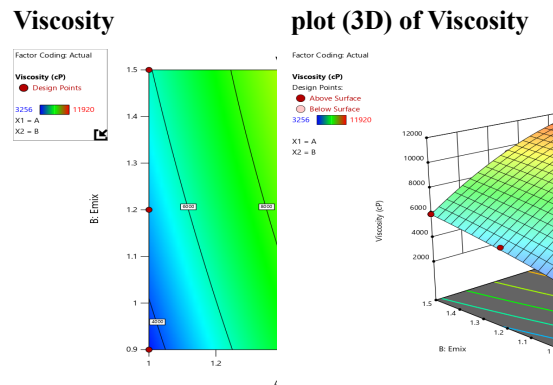
$$\text{Drug release} = 102.197 - 3.64333A - 0.138889B$$

Across all models, increasing Carbopol (A) and emulsifier (B) significantly enhances viscosity via a quadratic relationship. Conversely, both factors negatively impact spreadability and drug release, as higher component concentrations create a denser gel matrix that restricts flow and drug diffusion.

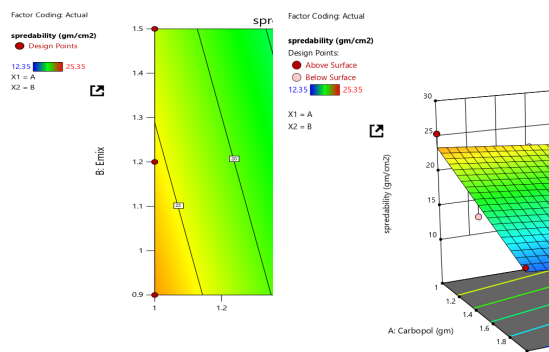
Contour and Response-surface Plots

The plots in Figure 4 show the same pattern in a visual form. The viscosity surface (Figure 4a) climbs steeply along the Carbopol axis, flattens slightly at the highest Carbopol level because of the curvature term, and rises only gently along the emulsifier axis. For spreadability (Figure 4b,c) the contour lines run mainly across the Carbopol axis, and the surface falls from low to high Carbopol, with a small additional decline as emulsifier increases. For drug release (Figure 4d,e) the contour lines are almost vertical, which means that release was governed nearly completely by Carbopol and was practically unchanged by the emulsifier.

a) Contour plot of Viscosity b) Response surface plot (3D) of Viscosity



c) Contour plot of Spreadability d) Response surface plot (3D) Spreadability



e) Contour plot of drug release f) Response surface plot (3D) drug release

Figure 4: Response-surface and contour plots for the 3² design: (a) viscosity contour (b) viscosity surface, (c) spreadability contour, (d) spreadability surface, (e) drug release contour and (f) drug release surface. A, Carbopol 940 (g); B, emulsifier (Emix).

Optimization and Validation

The factors were kept within the studied range (Carbopol 1–2%, emulsifier 0.9–1.5%), and numerical optimization proposed Carbopol 1.43% and emulsifier 1.33% (Table 6). The optimized batch was prepared and tested. It showed a viscosity of 8272 ± 253 cP, a spreadability of 18.2 ± 0.74 g·cm/s, a drug release of $97.49 \pm 0.39\%$ at 8 h and a pH of 6.5 ± 0.06 . The observed values differed from the predicted ones by -7.6% for viscosity, $+3.2\%$ for spreadability and $+0.7\%$ for drug release. All differences were below 10%, which supports the validity of the models for predicting the behaviour of the emulgel [31].

Table 6: Optimized leaf emulgel (TOLEF): predicted and observed responses

Response	Predicted value	Observed value (mean $\pm$ SD)	Difference (%)
Viscosity (cP)	8949.51	8272 ± 253	-7.6
Spreadability (g·cm/s)	17.63	18.2 ± 0.74	$+3.2$
Drug release at 8 h (%)	96.79	97.49 ± 0.39	$+0.7$

The optimized formulation was prepared from the obtained solution value from the numerical optimization. Optimized composition: Carbopol 940, 1.43% w/w; emulsifier mixture, 1.33% w/w.

Release Kinetics

The release data of the optimized emulgel were fitted to four models (Table 7, Figure 5). The Higuchi model gave the highest R^2 (0.9928), followed by the zero-order model (0.9808), the Korsmeyer–Peppas model (0.9522) and the first-order model (0.8416). The Higuchi equation describes release controlled by diffusion out of a matrix [27], which suits a fraction that must leave the oil droplets and move through the gel network. The Korsmeyer–Peppas model is generally used to judge the release mechanism from its exponent. As the zero-order R^2 is also high, the release may be close to steady over the studied period, and more sampling points would help to separate the two models.

Table 7: Regression coefficients (R^2) of the release kinetic models for the optimized emulgel (TOLEF)

Formulation	Zero order	First order	Higuchi	Korsmeyer–Peppas
TOLEF	0.9808	0.8416	0.9928	0.9522

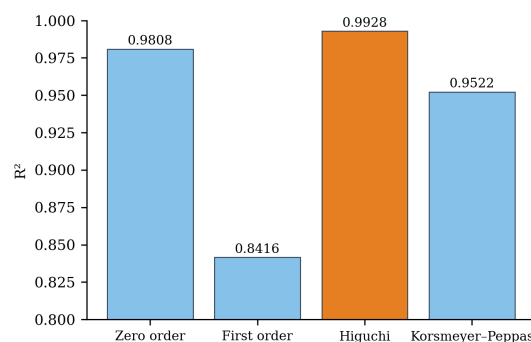


Figure 5: Regression coefficients (R^2) of the kinetic models for the optimized emulgel. The highest value is shown in orange

Skin Irritation

No erythema, oedema, ulceration, corrosion, necrosis or any other visible skin change was seen at any time during the 14-day observation period. The

emulgel was therefore well tolerated on rat skin under semi-occlusive conditions. This is in line with the near-neutral pH and the low level of emulsifier used, but the result applies only to this animal model and to the tested exposure.

Collagenase Inhibition

The leaf emulgel inhibited collagenase in a concentration-dependent way (Table 8, Figure 6), as reported for other plant extracts [6,7]. Inhibition rose from 7.74% at 20 $\mu\text{g/mL}$ to 65.28% at 100 $\mu\text{g/mL}$, and the IC_{50} value was 75.24 $\mu\text{g/mL}$. The standard, EDTA, rose from 29.37% to 86.11% over the same range, with an IC_{50} of 50.87 $\mu\text{g/mL}$. On an IC_{50} basis the leaf emulgel therefore had about two-thirds of the potency of the standard. This is a good result for a crude fraction placed in a semi-solid base, because the active constituents make up only a small part of the product. EDTA acts by binding the metal ion needed by the enzyme, and flavonoids have been linked with anti-ageing actions on skin [4,11,12], so the activity of the emulgel may come from the flavonoid content of the fraction, although this was not tested here. The activity of the plain fraction and of the placebo emulgel was not measured, and it is not yet possible to say how much of the effect is due to the fraction alone.

Table 8: Collagenase inhibition by the standard (EDTA) and the leaf emulgel (control absorbance at 335 nm = 1.68)

Concentration ($\mu\text{g/mL}$)	EDTA: mean absorbance	EDTA: inhibition (%)	Leaf emulgel: mean absorbance	Leaf emulgel: inhibition (%)
20	1.19	29.37 $\pm$ 1.50	1.55	7.74 $\pm$ 1.79
40	0.95	43.45 $\pm$ 1.19	1.34	20.04 $\pm$ 1.50
60	0.69	59.13 $\pm$ 2.09	1.12	33.13 $\pm$ 1.50
80	0.45	73.21 $\pm$ 1.79	0.76	54.76 $\pm$ 1.19
100	0.23	86.11 $\pm$ 1.50	0.58	65.28 $\pm$ 0.69
IC_{50} ($\mu\text{g/mL}$)		50.87		75.24

Inhibition is shown as mean $\pm$ SD of three replicates, calculated from the individual readings. IC_{50} values are those obtained from the concentration–response data.

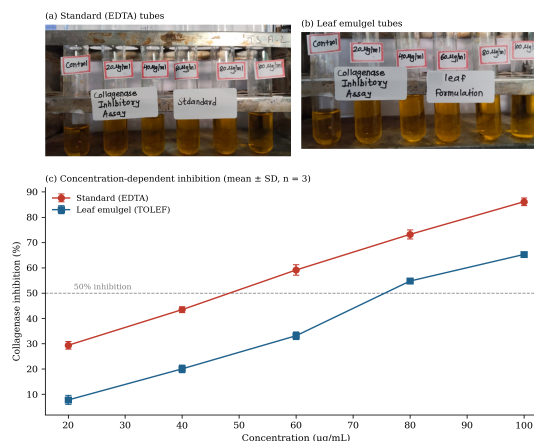


Figure 6: Collagenase inhibitory assay: (a) tubes with the standard (EDTA) and (b) tubes with the leaf emulgel at 0 (control) and 20–100 $\mu\text{g/mL}$; (c) concentration–response curves (mean $\pm$ SD, n = 3).

Stability

The emulgels stayed white, with no change in colour, homogeneity or phase separation, during 6 months at 40 $^{\circ}\text{C}$ and 75% RH (Table 9, Figure 7). For TOLEF, the pH moved from 6.5 to 6.3, the viscosity from 9471 to 9352 cP (a change of about 1.3%), the spreadability from 17.6 to 17.7 $\text{g}\cdot\text{cm/s}$ and the drug release from 98.5% to 98.03%. The

above observations indicate that the formulation has good stability under the experimental conditions.

Table 9: Stability of the optimized leaf (TOLEF) emulgels at 40 ± 2 °C and 75 ± 5% RH (mean ± SD)

Emulgel	Time (months)	Appearance	pH	Viscosity (cP)	Spreadability (g·cm/s)	Release at 8 h (%)
TOLEF	0	White	6.5 ± 0.12	9471 ± 89.63	17.6 ± 0.31	98.5 ± 0.19
	3	No change	6.5 ± 0.06	9468 ± 45	17.8 ± 0.67	98.4 ± 0.30
	6	No change	6.3 ± 0.06	9352 ± 69.82	17.7 ± 0.51	98.03 ± 0.32

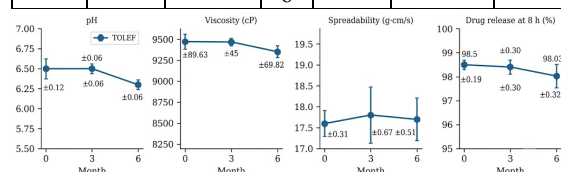


Figure 7: Changes in pH, viscosity, spreadability and drug release of TOLEF during 6 months at 40 °C and 75% RH (mean ± SD).

Conclusion

A topical emulgel incorporating the quercetin enriched fraction of *Thuja orientalis* was successfully developed and optimized using a 3² factorial design. Optimization outcomes identified Carbopol 940 as the primary factor governing viscosity, spreadability, and drug release, whereas the emulsifying mixture predominantly influenced system viscosity.

The optimized formulation—consisting of 1.43% Carbopol 940 and 1.33% emulsifier—exhibited experimental values that closely matched mathematical predictions, followed a Higuchi-driven diffusion release pattern, and demonstrated complete dermal safety with no signs of irritation in animal studies. Additionally, the emulgel maintained excellent physical and chemical stability over six months of accelerated storage conditions and exhibited notable, concentration-dependent anti-collagenase efficacy. These findings collectively highlight the potential of this delivery system as a

robust and effective topical vehicle for future anti-aging cosmeceutical and therapeutic applications.

Abbreviations

ANOVA: Analysis of Variance; cP: Centipoise; df: Degrees of Freedom; EDTA: Ethylenediaminetetraacetic Acid; FALGPA: N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala; FTIR: Fourier-Transform Infrared Spectroscopy; IC₅₀: Half-Maximal Inhibitory Concentration; ICH: International Council for Harmonisation; KBr: Potassium Bromide; NMR: Nuclear Magnetic Resonance; PTP1B: Protein Tyrosine Phosphatase 1B; q.s.: Quantity Sufficient; R²: Coefficient of Determination; RH: Relative Humidity; SD: Standard Deviation; TLC: Thin-Layer Chromatography; TOLEF: *Thuja orientalis* Leaf Emulgel Formulation; UV: Ultraviolet; w/w: Weight by Weight.

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