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Research article

Total Flavonoid Content, Antioxidant Properties, and COX Inhibition of a Dual-Extract Herbal Blend: Formulation of a Gel for Anti-Inflammatory and Wound-Healing Applications

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Abstract: The current work examined UDASP-HB, a novel herbal formulation comprising Urtica dioica methanol leaf extract and Angelica sinensis polysaccharide, for its pharmacological activity. It examined the total flavonoid content and its antioxidant and anti-inflammatory activity. UDASP-HB exhibited high concentrations of flavonoids, with considerable inhibition of lipid peroxidation in egg yolk and liver homogenate model compared with controls. UDASP-HB inhibited COX-1 and COX-2 enzymes and, therefore, can exhibit anti-inflammatory activity. Additionally, six new topical gel formulations and preparations were developed and examined for their physicochemical properties and in vitro drug delivery studies. All six preparations facilitated sustained delivery and effective dermal delivery.

Keywords: *Urtica dioica; Angelica sinensis*; Flavonoid content; *Angelica sinensis* polysaccharide; Lipid peroxide inhibition; Antioxidant activity

1. Introduction

Oxidative stress and inflammation represent two crucial biological processes with critical functions in cellular signaling and protection, but with the potential for chronic disease when not properly regulated. Oxidative stress occurs when a disproportion between ROS production and detoxification, and/or restoration of generated damage, takes place in a living organism [1, 2]. Inflammation, a physiologic reaction to noxious stimuli present in all tissues, can become persistent and disease-producing, as seen in conditions such as arthritis, diabetes, and cardiovascular disease. Regulation of such processes is not only critical for acute diseases, but also for long-term diseases, including degenerative diseases [1, 3, 4]. The modulating effects of oxidative stress and inflammation are well-established in traditional medical practice and are increasingly supported by scientific experiments. Application of such drugs is preferred for their lesser side effects, availability, and economy over conventional drugs [5-7].

Medicinal herbs contain a diverse range of bioactive compounds with high antioxidant and anti-inflammatory properties. Such compounds can trap free radicals, enhance antioxidant defense in the organism, and modulate inflammatory processes, thereby conveying protection against a variety of oxidative and inflammation-related ailments [1, 7-10].

Urtica dioica, or stinging nettle, has been used in traditional cultures, such as Ayurveda and Western herbalism, for centuries to control disease processes including arthritis, anaemia, and long-term dermatological conditions [11-13]. All its therapeutic efficacy can be attributed to its rich content of phytochemicals such as flavonoids, lignans, and polysaccharides. *Angelica sinensis*, popularly known as Dong Quai or female ginseng, is famous in traditional Chinese medicine for its use in treating gynaecologic diseases, weakness, mild anaemia, and high blood pressure. Polysaccharides of *Angelica sinensis* (ASP) have been shown to possess strong anti-inflammatory and immunomodulatory properties, making them a beneficial constituent in herbal drugs for wound healing and anti-inflammatory activity [2, 14].

The premise for developing a herbal mixture of *Urtica dioica* leaf methanol extract and polysaccharide of *Angelica sinensis* (ASP) for the development of herbal gels is the synergistic effect between their bioactivities, enhancing anti-inflammatory activity and wound healing. Complementarily, it leverages *Urtica dioica's* high anti-inflammatory activity and ASP's proven wound healing ability [15, 16]. Such a preparation not only aims to counteract the processes of inflammation but also to accelerate tissue regeneration and healing processes [15]. Gels, when locally applied, have the added advantage of localized delivery, which can maximize therapeutic activity at the site of trauma and inflammation while minimizing systemic toxicity. In harmony with a growing demand for effective and natural substitutes for traditional drugs in controlling trauma and inflammation and healing wounds, such an approach holds a lot of hope for future development in herbal therapy [17].

Therefore, the purpose of the current study is to investigate the pharmacological potential of a methanol extract derived from *Urtica dioica* leaves with a microwave-assisted extraction process. The pharmacological potential of *Angelica sinensis* polysaccharides is another goal of the investigation. Additionally, the study aims to develop a topical herbal gel formulation using an herbal blend of *Angelica sinensis* polysaccharide and *Urtica dioica* methanol leaf extract. Building on their traditional uses and earlier research, these historically prized medicinal plants will be evaluated in various models to determine their effects on inflammation and oxidative stress. The purpose of this study is to advance our understanding of its therapeutic applications and encourage its potential integration into contemporary medical procedures.

2. Materials and Methods

2.1 Drugs, Chemicals, Polymers, and Reagents

Zen Pharmaceuticals, Karnal, India, generously supplied free samples of Catechin and quercetin. Himedia Biosciences Company in India provided the following: Triethanolamine, disodium edetate, DTNB solution (Ellman's reagent), sodium nitroprusside, Griess Reagent, thiobarbituric acid, sodium dodecyl sulphate, and tris-KCl buffer. The trichloroacetic acid (TCA) was purchased from Sigma Aldrich in India. Additionally, Sigma-Aldrich in India provided propylene glycol, Carbopol 934, and Carbopol 940. All additional chemicals and reagents utilised in this investigation, bought from verified vendors such as SRL Mumbai and E. Merck India, were of reagent grade and ensured high-quality standards.

2.2 Plant material collection and authentication

Between November 2023 and February 2024, *Urtica dioica* leaves were carefully collected from their natural environment to ensure a steady supply of mature and healthy plant material. The polysaccharide of *Angelica sinensis* was sourced from Herbal Waves in Mandi, Himachal Pradesh, India. During leaf collection, special attention was paid to preserving the *Urtica dioica* habitat in its natural setting. A local herbalist named Mr. Ashok Gupta, who works with Herbal Waves in Himachal Pradesh, India, verified and identified the samples after they were harvested. During the authenticity stage, voucher samples stored in the Department of Pharmacognosy Herbarium (MSK/ACP/2023/3398-72) were carefully examined. This meticulous analysis ensured that the plant material under study was appropriately classified through taxonomy.

2.3 Preparation of extraction utilizing a microwave-assisted method

The collected *Urtica dioica* leaves were gently washed to remove contaminants, such as dust and grime. The cleansed leaves were then dried in a shaded area to preserve the integrity of the phytochemicals and prevent deterioration from direct sunlight. The leaves were dried and then ground into a fine powder using a motorised grinder to achieve consistency in particle size for efficient extraction. According to Mandal et al. (2007), the extraction method employed a microwave-assisted extraction approach [18]. Twenty-five grams of the powdered leaves were combined with 200 millilitres of methanol in a 1:8 powder-to-solvent ratio. Microwave irradiation was applied to the mixture with a power setting of 160 W. During the first six minutes of the extraction procedure, the mixture was heated to the optimal temperature for maximum extraction. To ensure the full extraction of bioactive components, the temperature was then allowed to drop to room temperature before initiating a second 2-minute microwave cycle. Particulate materials and plant debris were eliminated from the resultant extract using filtering. After the solvent was removed from the filtrate using a water bath, a dense and concentrated extract was produced. *Angelica sinensis* polysaccharide (ASP) was subsequently added to the finished extract in a 1:1 ratio. The final herbal blend was subsequently codenamed UDASP-HB and stored for future testing.

2.4 Determination of total flavonoid content

A spectrophotometric aluminum chloride technique, described earlier with minor modifications, was utilized for the determination of the total flavonoid content of UDASP-HB [19]. Aluminium chloride ($AlCl_3$), which exhibits a detectable absorption peak at a specific wavelength with flavonoids, was used in this protocol. Flavonoid contents were calculated in terms of milligrams of equivalent quercetin (mg QE/g) per gram of extract in a plant. In a 1-mL UDASP-HB solution (1 mg/mL), 1 mL and an equivalent (1 mL) 2% methanolic solution of $AlCl_3$ were mixed in a test tube to form a sample for analysis. After a brief vortex to achieve homogeneity, the mixture was kept at room temperature for 1 hour to allow the reaction between the flavonoids and $AlCl_3$ to proceed to completion. After incubation, the absorption at 415 nm was measured using a Shimadzu UV-1900 spectrophotometer in a solution. Since 415 nm is equivalent to the absorption at a maximum for the $AlCl_3$-flavonoid complex, it was used to enable proper determination. All samples were prepared and analyzed three times for repeatability and accuracy. Additional computations were performed using the mean values for absorption in these samples. In a similar manner, a series of solutions with a range of known concentrations of quercetin was used to obtain a standard curve. A calibration curve, used as a guideline for determining the extract level of flavonoids, was produced by plotting the values for absorption of these standards concerning their respective concentrations. Value for absorption of the sample was interpolated from the curve for the standards for quercetin in an attempt to calculate UDASP-HB level of flavonoids. The quantitation of the extract level of flavonoids was provided in the output, expressed in terms of equivalent milligrams of quercetin per unit extract per gram (mg QE/g). It is an efficient and effective method for determining flavonoids in plant samples.

2.5 Evaluation of the lipid peroxidation inhibition using egg yolk and liver homogenate model

Using egg yolk as a lipid-rich medium and a modified thiobarbituric acid reactive species (TBARS) assay, the amount of lipid peroxide product generated was quantified [20]. Using egg yolk/liver homogenate as a source of high fat, inhibition of lipid peroxidation was examined using a modified thiobarbituric acid reactive species (TBARS) assay, according to Badmus et al. (2011). By testing for malondialdehyde (MDA) production, a significant metabolite of lipid peroxides, through its reaction with thiobarbituric acid (TBA), analysis for inhibition in lipid peroxidation is performed. As a substrate for lipids, 0.7 mL of 14% (v/v) egg yolk/liver homogenate emulsion and 0.5 mL of extract (UDASP-HB) at variable concentrations (15–180 μg/mL) were mixed to make a reaction mixture. To create a uniform reaction mixture, 1.6 mL of distilled water was added. Lipid peroxidation was initiated by incubating the mixture with 0.06 mL of a freshly prepared ferrous sulfate ($FeSO_4$) solution. Incubation of the reaction mixture at 37°C for 35 minutes provided a sufficient duration for oxidative degradation of the lipids. Following incubation, 1.7 mL of acetic acid and 1.8 mL of TBA in sodium dodecyl sulphate (SDS) solution were added to it. TBA and MDA react readily in an acidic environment to form a pink-colored chromogen, the presence of which can be determined using a spectrophotometer. After shaking the mixture well to achieve homogeneity, it was placed in a water bath and incubated for 1 hour at 95°C. Heating helps in intensifying the reaction between TBA and MDA. For the extraction of the TBA-MDA complex into an organic layer, 6 mL of butanol was added when the mixture was

cooled to room temperature. For phase partitioning, the mixture was centrifuged for 15 minutes at 4000 rpm. TBA-MDA complex-containing organic upper layer was collected with extreme care, and a UV-visible spectrophotometer was used to determine its absorption at 532 nm.

$$\%\ \text{Lipid peroxidation inhibition } \left(\frac{100 - X_{SAMPLE}}{X_{CONTROL}}\right) \times 100$$

2.6 Appraising the Anti-inflammatory activity: Cyclooxygenase (COX) inhibition assay

The assay for Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) enzymes was conducted using the protocol described elsewhere [21, 22]. The UDASP-HB extract's cyclooxygenase (COX) inhibitory activity was measured through COX-1 and COX-2 enzyme tests following protocols designed by Redl et al. (1994) and Aguilar et al. (2002). Inhibition of COX-1, an enzyme responsible for prostaglandin synthesis for a normal physiologic function, was measured through the COX-1 test. 195 µL of 0.4 M Tris-HCl (pH 7.4), 20 µL of L-adrenaline-D-hydrogentartrate working as a reductant, 15 µL of haematin working as a cofactor, and 15 µL of a sample solution were added to the reaction mixture before starting the experiment. 0.4 U of COX-1, added later, initiated incubation for 7 minutes at 37°C to initiate the reaction in the enzyme. 7 µL of arachidonic acid, serving as a substrate, was added later, and the mixture was incubated at 37°C for 35 minutes to initiate the reaction. The addition of 15 µL of 14% formic acid later inhibited the enzyme's activity. The COX pathway produced prostaglandin E2 (PGE2), and its activity level was determined using a PGE2 enzyme immunoassay kit (R&D Systems). Likewise, in the COX-2 study, a similar sequence of operations was conducted to assess the inhibition of COX-2 by UDASP-HB, an enzyme with a predominantly causative role in producing inflammation. In its reaction mixture, 15 µL of sodium edetate (Na_2-EDTA) was added to chelate metal ions, 15 µL of haematin, 20 µL of L-adrenaline-D-hydrogentartrate, 195 µL of 0.4 M 7.4-pH Tris-HCl, and 15 µL of a sample solution were added. After the addition of 0.4 U of COX-2 enzyme, the mixture was incubated for 6 minutes at 37°C. After the addition of 7 µL arachidonic acid substrate, a 35-minute reaction at 37°C, and a 35-minute reaction duration, 14 µL of 14% formic acid was added, which inhibited its activity. In its determination, the generated PGE2 level in the COX-2 pathway was measured using a PGE2 enzyme immunoassay kit (Cayman Systems) [21, 22]. For accuracy and repeatability, both tests were conducted in triplicate. By comparing the results with and without the extract blend, the inhibition level of PGE2, the inhibition of COX activity, and the percentage inhibition of COX activity were calculated. By employing this technique, a comprehensive analysis of the inhibition of the extract on COX-1 and COX-2 enzymes could be conducted.

2.7 Formulation development: The fabrication of the herbal gel

2.7.1 Preparation of the gel base

The gel base preparation involved careful homogeneity and uniformity in any preparation form. In preparation, 1.5 g of Carbopol 934 was added first under slow and continuous stirring in such a form that no clumps and uniformity in form existed. It was a key stage in hydrating the polymer and creating a uniform dispersion, a crucial property for achieving uniformity in the finished form, as well as its stability and shape. A triethanolamine and disodium EDTA solution was prepared in a separate form after hydrating the Carbopol. To maintain chemical integrity in the gel, 0.005 g of disodium EDTA was utilized in a chelating and chemical integrity-maintaining role, and 1.5 g of triethanolamine was used in a pH-adjusting and neutralizing role. To allow proper solubilization, both chemicals were vigorously mixed in a combined form with deionized water. Following continuous stirring, the drop-by-drop addition of triethanolamine and disodium EDTA, and slow, drop-by-drop blending, a transparent and thick gel matrix is developed in its presence, with a pH level of about 7.4 before addition. This critical stage offers an opportunity for Carbopol neutralization. Similarly, 5.5 g of propylene glycol, a humectant and solvent, was added to moisturize and strengthen the gel, providing a transparent, uniform base for the developed gel. This was achieved by blending all ingredients under continuous stirring for proper integration [23].

2.7.2 Preparation of gel formulation

The drug formulation manual was followed in the preparation of a total of six topical gel formulations [24]. Following base gel preparation, six individual gel preparations, HGF1 to HGF6, were prepared with

variable UDASP-HB concentrations (a mixture of *Urtica dioica* methanol leaf extract and *Angelica sinensis* polysaccharide). UDASP-HB concentrations ranged in gradation, starting with 0.5 g in HGF1 and increasing by 0.5 g in each successive preparation, with a final concentration of 3.0 g in HGF6. One could evaluate the impact of a mixture of polysaccharides on the therapeutic activity of the gel with such a gradation. To ensure a uniform proportion of basis materials in each preparation, a portion of UDASP-HB was added to a base gel and brought to a 100 g weight with a mixture of demineralized water. In terms of anti-inflammatory activity and wound healing, each batch was properly prepared for future testing and therapeutic application by using a consistent preparation for both the base and subsequent formulations.

Table 1. The list of components used in the formulations of herbal gels.

	Gel Formulation code					
Ingredients	**HGF1**	**HGF2**	**HGF3**	**HGF4**	**HGF5**	**HGF6**
UDASP-HB (g)	0.5	1.0	1.5	2.0	2.5	3.0
Carbopol 934 (g)	1.5	1.5	1.5	1.5	1.5	1.5
Triethanolamine (g)	1.5	1.5	1.5	1.5	1.5	1.5
Disodium EDTA (g)	0.005	0.005	0.005	0.005	0.005	0.005
Propylene Glycol (g)	5.5	5.5	5.5	5.5	5.5	5.5
D.M. water (100 g)	q.s	q.s	q.s	q.s	q.s	q.s

2.8 Characterization of the gel formulation

2.8.1 Assessment of active constituents

To precisely identify the phenolic contents as an assay method for the active elements in the herbal gel preparation, effective techniques were used, including total phenolics analysis using the Folin-Ciocalteu method. A 1 g portion of the gel was taken in a 50 mL volumetric flask, and methanol was added to make a solution for the active contents. Methanol and the flask contents were mixed vigorously to form a homogeneous solution of the contents in methanol. Whatman filter paper was then used to remove any residues that did not dissolve in methanol. 0.1 mL of filtrate was measured with care and diluted to a final volume of 10 mL with methanol to form a testing solution for analysis. Concentrations of active contents in such a solution are calculated using two approaches with the use of UV spectrophotometry at a proper wavelength, a wavelength at λmax for active compounds [25]. After preparing the herb gel in a mixture with ethanol or methanol, a determined quantity of Folin-Ciocalteu reagent was added, and the mixture was incubated for around ten minutes. Blue colour development was carried out for ninety minutes after sodium carbonate was applied for neutralisation. The absorbance was measured at 765 nm. A gallic acid standard curve was used to quantify the phenolic chemicals.

2.8.2 Extrudability

An essential factor for user convenience, the gel formulation's extrudability was evaluated to determine how easily it could be extruded from the container. A common weight (such as 500 g) was placed on top of the slide after a collapsible aluminium or plastic tube carrying the gel was sandwiched between two glass slides. Weighing was performed to determine the amount of gel extruded in 10 seconds. The amount of gel extruded per unit weight applied was used to express the extrudability of the gel. This test confirmed that the gel had the ideal consistency for easy and smooth dispensing [26].

2.8.3 pH Measurement

To minimize irritation during use and ensure compliance with the skin's natural pH, the pH of the gel preparation was determined. For the preparation of a uniform solution, a small portion (1 g) of gel was blended with 10 milliliters of deionized water. A calibrated electronic pH meter with an inserted electrode was used to measure and scan the pH value of the gel dispersion. For use in derms, its value in the acceptable range (typically 6.8–7.5) was reached, and a value of 7.4 was maintained throughout the entire formulative exercise [27].

2.8.4 Evaluation of appearance, viscosity, and homogeneity

Appearance

A visual examination was performed to evaluate the transparency, color, and overall cosmetic properties of the gel preparation. Consistency in transparency and colour between the two sets of the gel was examined to ensure compliance with the desired specifications for use in a topically applicable preparation [28].

Viscosity

The viscosity of the gel preparation was measured using a Brookfield viscometer to assess its rheological properties. The spindle (which can be used for semi-solid preparations) was subjected to variable velocities (in rpm) at room temperature with the gel loaded onto the viscometer. To analyze the flow behavior of the gel, viscosity values were measured at a range of velocities. Shear-thinning or pseudo-plastic behavior is ideal for a gel, allowing for spreadability and ease of application while maintaining long-term stability. To maximize dermal application consistency of the gel, such viscosity determination was imperative [28].

Homogeneity

To assess the homogeneity of the gel, a small portion of the mixture was left on a sanitized glass slide and inspected under a light source. We searched for lumps, clumps, and particulate matter in the gel. The easy and unhindered dispersion of the ingredients suggested that the gel would be successfully prepared and properly mixed [28].

2.8.5 Spreadability

One important property that controls the ease with which the gel can penetrate and cover the skin is its Spreadability. Spreadability controls both happiness and comfort in use, as well as the therapeutic effectiveness of a preparation, directly. The spreadability of a gel was measured using a slide and drag technique. In it, two flawless plates of glass were placed between a definite amount of gel (e.g., 1 g). For allowing the spreading of a gel, a uniform weight (500 g, for example) was kept on a top plate for a definite duration, such as one minute. After removing the weight, the diameter or cover area was measured. Spreadability of a formula was calculated through:

$$S = M \times L / T$$

where:

- S is the spreadability,
- M is the weight applied (in grams),
- L is the length of gel spread (in cm),
- T is the time taken (in seconds).

Higher values for spreadability in the form of a gel mean easier and uniform application onto the skin's surface. In testing, care is taken to ensure the gel is effective and convenient for topical distribution due to its uniform dispersibility, requiring minimal effort [29].

2.8.6 In vitro Diffusion study evaluating the permeation through skin

Goat ear skin was then used in *in vitro* diffusion experiments to evaluate its capacity to penetrate through dermal tissue and simulate transdermal distribution. Goat ear skin for use in experiments was harvested at a nearby slaughterhouse, and peeling off of the dermal tissue was handled with care, with consideration for keeping the dermal tissue intact. After peeling off fat and subcutaneous tissue, saline was used to wash off the dermal tissue, and it was stored at -20°C for use as needed. Before use in experiments, the dermal tissue was incubated in phosphate buffer (pH 7.4) for one hour at room temperature. A Franz diffusion cell with a receptor compartment volume of 15 mL and an effective diffusion area of 2.5 cm^2 was used in the study. With the stratum corneum towards the donor side and the dermis in touch with the receptor medium, the hydrated skin was positioned between the donor and receptor compartments. The receptor medium was phosphate buffer (pH 7.4), which was maintained at 37 ± 1°C and stirred constantly to replicate physiological conditions. The donor compartment was uniformly coated with a predetermined quantity of the gel formulation (about 1 g). At specified intervals (e.g., 0, 1, 2, 4, 6, 8, and 12 hours), samples (1 mL) were removed from the receptor compartment and replaced with an equivalent volume of new phosphate buffer to preserve sink conditions. The active ingredients that penetrated the skin were measured by spectrophotometric analysis of the collected samples as described earlier in the assay method. The permeation profile was generated by plotting the cumulative amount of penetrated drug per unit area ($\mu g/cm^2$) against time. The linear portion of the permeation curve was used to determine the steady-state flux (Jss), and the flux

was correlated with the active ingredient in the donor compartment to determine the permeability coefficient (Kp). In the present work, it facilitated the optimization of the gel's form for effective transdermal delivery and therapeutic activity by providing useful information about its penetration and release behavior in well-regulated *in vitro* environments. A goat ear membrane was used as a model to evaluate the function of the gel in closely monitored *in vitro* environments [29].

2.8.7 Release Kinetics of Gel Formulation

To evaluate the kinetics of release of the active components from the gel preparation, an in vitro Franz diffusion cell setup was used with phosphate buffer (pH 7.4) at 37 ± 1°C. A specific quantity of gel in the donor chamber and samples extracted from the receiver medium at regular intervals are necessary to maintain sink conditions. The volume was withdrawn, and a fresh buffer was added. Samples were examined using UV-Vis spectrophotometry [30]. To ascertain the release mechanism, cumulative release data were plotted against time and examined using mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The model with the highest correlation coefficient best represented the release kinetics. This procedure verified that the gel formulation offered sustained, controlled release, which is necessary for its therapeutic effectiveness and directs further optimisation for the required drug delivery profiles.

2.9 Statistical analysis

Statistical analysis was conducted to ensure the reliability and reproducibility of experimental results. All experiments were performed in triplicate, with data expressed as mean ± standard deviation (SD). For significance testing between groups, one-way ANOVA followed by Tukey's *post hoc* tests was used for multiple groups, and independent t-tests were used for two groups, considering p-values less than 0.05 as statistically significant. Regression analysis was used to fit the release data to various kinetic models, evaluating model adherence through the correlation coefficient (r^2), with higher r^2 values indicating a better fit. All statistical procedures were executed using GraphPad Prism (Version 8), ensuring precision and efficiency. The programs' R and Python were used for graphing. This robust statistical framework validated the findings and supported interpretations of the gel formulations' efficacy and performance.

3. Results and Discussion

3.1 Total flavonoid content estimation

UDASP-HB was found to have a total flavonoid content (TFC) of 357.75 mg/g of quercetin equivalent (QE). The calculated absorbance values were used to estimate the flavonoid concentration using the calibration curve equation, y = 0.0031x + 0.0693, with R^2 = 0.9691. The significant flavonoid content of the extract emphasises its potential for antioxidant and therapeutic applications.

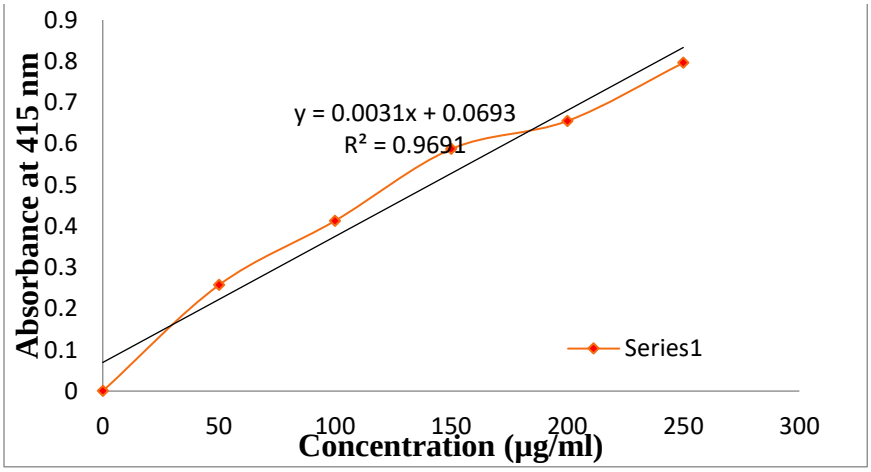

Figure 1. Quercetin standard curve to estimate total flavonoid concentration in UDASP-HB.

3.2 Inhibition of Lipid peroxidation in the egg yolk model

The inhibition activity of UDASP-HB on egg yolk model lipid peroxidation at varying concentrations, compared to ascorbic acid, a well-known antioxidant, is presented in Table 2. UDASP-HB, at its highest under investigation concentration (180 μg/ml), showed a high inhibition value of 84.87 ± 1.26%, closely following that of ascorbic acid, whose inhibition value at 95.39 ± 1.22% was marginally increased. With a decrease in UDASP-HB concentrations, its inhibition activity for egg yolk model lipid peroxidation consistently lowered: 75.68 ± 1.28% at 90 μg/ml, 67.91 ± 1.28% at 60 μg/ml, 56.82 ± 1.22% at 30 μg/ml, and 44.71 ± 1.14% at 15 μg/ml. Ascorbic acid, in its part, showed lowered inhibition with a decrease in concentrations, but at a level higher than UDASP-HB at all concentrations under investigation. That behaviour indicates UDASP-HB's dose-dependent effectiveness, which is noteworthy for its antioxidant activity, although it is even less potent than that of ascorbic acid. Notably, even at the minimum level (15 μg/ml), UDASP-HB exhibited a significant inhibition percentage, confirming its effectiveness as a powerful antioxidant.

Table 2. In the model of egg yolk homogenates, the percentage of lipid peroxidation inhibition of UDASP-HB

Media	Concentration (μg/ml)	UDASP-HB (%)	Ascorbic acid (%)
Egg yolk	180 μg/ml	84.87 ± 1.26*	95.39 ± 1.22*
	90 μg/ml	75.68 ± 1.28*	86.64 ± 1.90*
	60 μg/ml	67.91 ± 1.28*	80.49 ± 1.78*
	30 μg/ml	56.82 ± 1.22*	62.24 ± 1.67*
	15 μg/ml	44.71 ± 1.14*	56.54 ± 1.25

Values are expressed using the standard deviation (SD) plus the mean of three replicate measurements.
*$p < 0.05$ significant deviations from ascorbic acid.

3.3 Inhibition of Lipid peroxidation in liver homogenate model

Table 3 below shows the in vitro inhibition of LPO in liver homogenate at UDASP-HB doses of catechin, a potent antioxidant. At 180 μg/ml, UDASP-HB exhibited a high inhibition value of 91.54 ± 1.98%, indicating high antioxidant activity, albeit somewhat lower than the 98.88 ± 1.67% observed for catechin. The efficacy of UDASP-HB decreases as concentrations rise: 82.63 ± 1.93% at 90 μg/ml, 73.38 ± 1.41% at 60 μg/ml, plateaus at 72.91 ± 1.32% at 30 μg/ml, and then falls to its lowest value of 51.78 ± 1.22% at 15 μg/ml. Conversely, catechin exhibited a strong inhibitory effect at all concentrations, but a steep decline at lower concentrations, decreasing from 94.68 ± 1.69% at 90 μg/ml to 69.84 ± 1.37% at 30 μg/ml. While catechin exhibits slightly higher antioxidant activity at comparable concentrations, the trend in this instance reflects the dose-dependent efficacy of both UDASP-HB and catechin. In terms of *$p < 0.05$ and **$p < 0.01$, statistical analysis demonstrates that the inhibition rate differences between UDASP-HB and catechin are significant at any level, but particularly at high concentrations. It also demonstrates that UDASP-HB is a potent antioxidant in its own right, even when it does not exhibit the same level of efficacy as catechin.

Table 3. *In vitro* evaluation of lipid peroxidation (LPO) inhibition using liver homogenate

Media	Concentration (μg/ml)	UDASP-HB (%)	Catechin (%)
Liver homogenate	180 μg/ml	91.54 ± 1.98**	98.88 ± 1.67**
	90 μg/ml	82.63 ± 1.93**	94.68 ± 1.69*
	60 μg/ml	73.38 ± 1.41*	83.91 ± 1.43*
	30 μg/ml	72.91 ± 1.32*	69.84 ± 1.37*
	15 μg/ml	51.78 ± 1.22*	62.59 ± 1.27*

Values are expressed as the standard deviation (SD) plus the mean of three replicate measurements. Differences from the Catechin group are significant at *$p < 0.05$ and **$p < 0.01$.

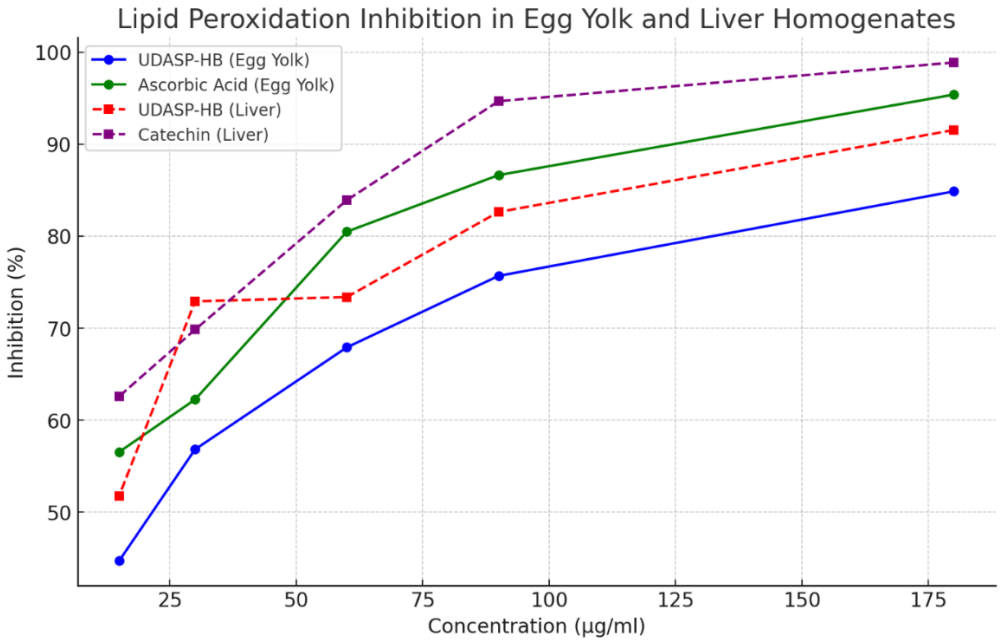

Figure 2. In vitro lipid peroxidation (LPO) Inhibition of UDASP-HB using egg yolk homogenates and liver homogenates

3.4 Evaluation of anti-inflammatory activity

3.4.1 Assessing the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme inhibition

The inhibitory activity of UDASP-HB, the herbal blend, towards COX-1 and COX-2 enzymes at the indicated concentrations is shown in Table 4. There is an increased inhibition in a concentration-dependent manner for both enzymes. At a concentration of 60 µg/mL, inhibition was relatively low, with 3.791±0.89% inhibition for COX-1 and a relatively increased inhibition for COX-2 at 4.678±0.96%. With a heightened concentration to 120 µg/mL, inhibition for COX-1 reached almost double at 6.272±0.97%, and for COX-2, inhibition rose over double at 10.366±0.99%, with a reflection of a strong impact towards COX-2 at this level. The trend continued with increased concentrations. In 180 µg/mL, COX-1 inhibition increased to 22.231±1.01%, and COX-2 inhibition increased to 27.821±1.21%. It is a significant trend, suggesting a deeper inhibition of COX-2, a target for anti-inflammatory drugs in most instances, due to its direct role in inflammatory processes. In its most extreme inhibition, at 240 µg/mL, COX-1 inhibition increased to 45.306±1.21%, and COX-2 inhibition increased to 44.793±1.77%. These observations suggest that UDASP-HB has significant therapeutic value as an anti-inflammatory agent, primarily through the inhibition of COX-2. Concentration-dependent activity creates opportunities for individualized dosing tailored to specific therapeutic objectives. Observations validate the need for continued investigation regarding UDASP-HB therapeutic use, specifically in cases of disease where COX-2 is a significant participant.

Table 4. Percentage of COX system enzymes inhibited by the herbal blend (UDASP-HB).

	% Enzyme Inhibition	
Concentration (µg/mL)	**COX-1**	**COX-2**
60	3.791 ± 0.89	4.678 ± 0.96
120	6.272 ± 0.97	10.366 ± 0.99
180	22.231 ± 1.01	27.821 ± 1.21
240	45.306 ± 1.21	44.793 ± 1.77
300	94.698 ± 1.67	86.576 ± 1.82
IC_{50}	233.21 µg/mL	229.89 µg/mL

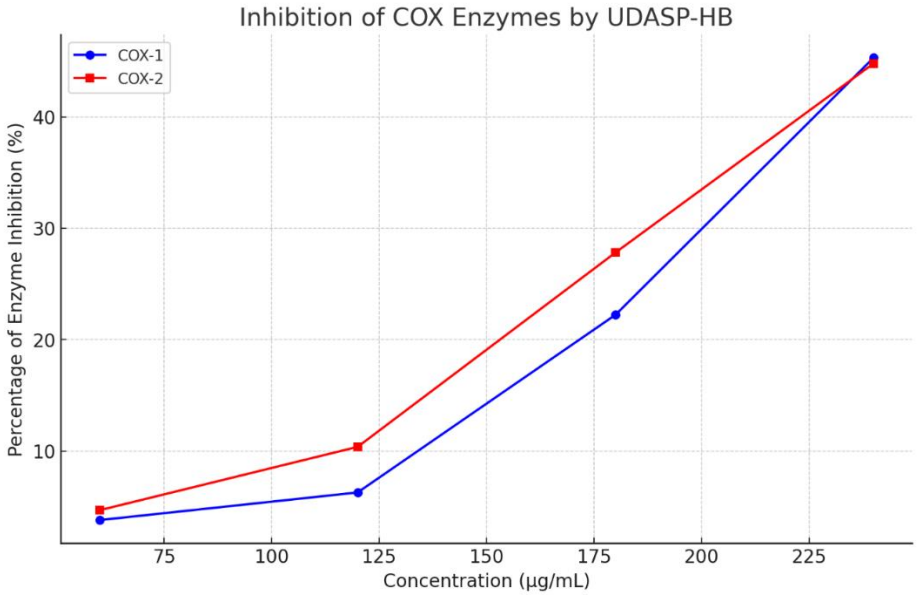

Figure 3. Percentage of COX system enzymes inhibited by the herbal blend (UDASP-HB).

3.5 Herbal Gel Formulation: development and evaluation of the topical herbal gel

Gel formulations are typically preferred over other topical semisolid treatments for several reasons. Their long-lasting effects on the skin, high viscosity, occlusive qualities that moisturise flaky skin, improved bioadhesiveness, decreased irritation, independence from the water solubility of active ingredients, ease of application, and improved release characteristics are just a few of their many benefits [31-33]. The anti-inflammatory, antioxidant, protective, and anti-aging properties of flavonoids and phenolic compounds found in herbs have been the subject of numerous studies. Furthermore, it has been shown that these polyphenolic flavonoids can permeate human skin [34-37]. These results led to the development of a topical herbal gel formulation that includes extracts high in flavonoids and phenolic components for the prevention and treatment of inflammation, wounds, and related disorders [38-40].

3.6 Fabrication and Characterisation for the Formulated Topical Herbal Gel

Table 5 presents the characteristics of various prepared topical gel formulations, each prepared with 1.5% Carbopol 934, highlighting differences in pH, viscosity, spreadability, and net content across six formulations coded HGF1 through HGF6.

3.6.1 pH Levels: The pH of all formulations is near neutrality (approximately 7.4), which is optimal for dermal use, as it closely corresponds to the in-situ skin pH and reduces irritation. A slight deviation in pH (ranging from 7.39 to 7.54) between the formulations is within a narrow range. It reflects a high level of uniformity in terms of the acid-base character of the formulation.

3.6.2 Viscosity: A definite rise in viscosity with HGF1 through HGF6, with a range of 4.5 poise through 9.4 poise, was seen. A rise in viscosity will likely result in a thicker gel structure, making it easier for the gel to maintain its placed position and not drip excessively. Viscosity can work beneficially when applied directly to the skin, particularly with a thicker level used for an extended duration of activity.

3.6.3 Spreadability: Correspondingly, with increased viscosity, spreadability also increases; for instance, 35.71 g/cm/s in HGF1 and 79.36 g/cm/s in HGF6. Spreadability is an important property for topical gels, describing how a gel spreads when it comes into contact with the skin. Greater spreadability will mean less force is required to spread the gel, providing increased comfort and efficiency in covering larger areas of skin.

3.6.4 Net Content: The net contents of all samples have remarkably similar 100% values, with little variation (98.96% to 101.00%). Such homogeneity in net contents ensures precise dosing, confidence in consumption, and superior manufacturing without overfilling or underfilling. In general, the improvement in viscosity and spreadability between HGF1 and HGF6 reflects that the formulations have been optimized for

application performance improvement, and thus can function even better for subjects with variable requirements for gel textures and spreading velocities. High accuracy in all formulations' net contents reflects a high level of care and accuracy in production.

Table 5. Characteristics of the topical gel formulations prepared with 1.5% Carbopol 934 Concentration.

Code	pH	Viscosity (poise)	Spreadability (g cm/sec)	Net Content (% w/w)
HGF1	7.41 ± 0.17	4.5 ± 0.11	35.71 ± 1.14	98.96 ± 1.45
HGF2	7.39 ± 0.18	5.5 ± 0.12	47.44 ± 1.34	99.95 ± 1.99
HGF3	7.54 ± 0.16	5.9 ± 0.13	58.81 ± 1.39	100.20 ± 1.94
HGF4	7.46 ± 0.19	7.2 ± 0.21	66.77 ± 1.45	101.00 ± 1.89
HGF5	7.51 ± 0.13	8.6 ± 0.18	73.62 ± 1.60	100.00 ± 1.85
HGF6	7.39 ± 0.11	9.4 ± 0.01	79.36 ± 1.72	99.91 ± 1.85

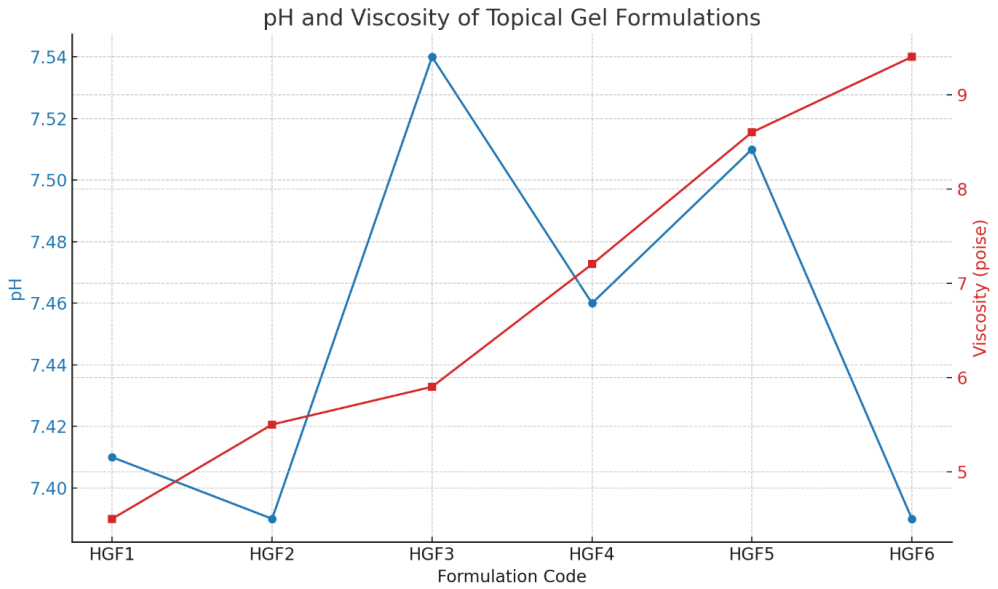

Figure 4. pH and Viscosity (poise) of Gel Formulations

3.6.5 Extrudability and Physical Appearance

The following is a general overview of the extrudability and physico-morphology of a range of topical gel forms, prepared at 1.5% w/w of Carbopol 934, coded HGF1 to HGF6, respectively.

Extrudability: One of its key functions is user-friendliness, which reflects how easily the gel can be extruded from its packaging. Since HGF1, HGF4, and HGF5 have "Excellent" extrudability, these gels should be quite easy to squeeze out, perhaps as a result of viscosity at the optimal level for unhindered free flow. Although they are effective, HGF2, HGF3, and HGF6 exhibit "Good" extrudability and less free flow than "Excellent."

Physical Appearance: All of these formulations have been uniformly described as dark greenish, transparent, and homogeneous in form. Homogeneity in form is an indicator of correct mixing during production, ensuring that no form separation or variation in colours occurs, which can be important for trust in consumption and product consistency. Transparency and homogeneity in form in the gels also mean that particulate impurities have been circumvented and successfully stabilized, which can be important for cosmetic appearances and uniformity in application over the skin. In inference, these observations in Table 6 validate the successful development of these topical gels, both functionally and cosmetically. Overall, good to excellent extrudability in all cases confirms ease of use and effectiveness during use, as well as a uniform, pleasing physical appearance that enhances marketability and acceptance, and promotes increased patient acceptance and use. All these, in addition to the physical characteristics mentioned in the preceding tables, contribute to the overall quality and therapeutic efficacy of these gel preparations.

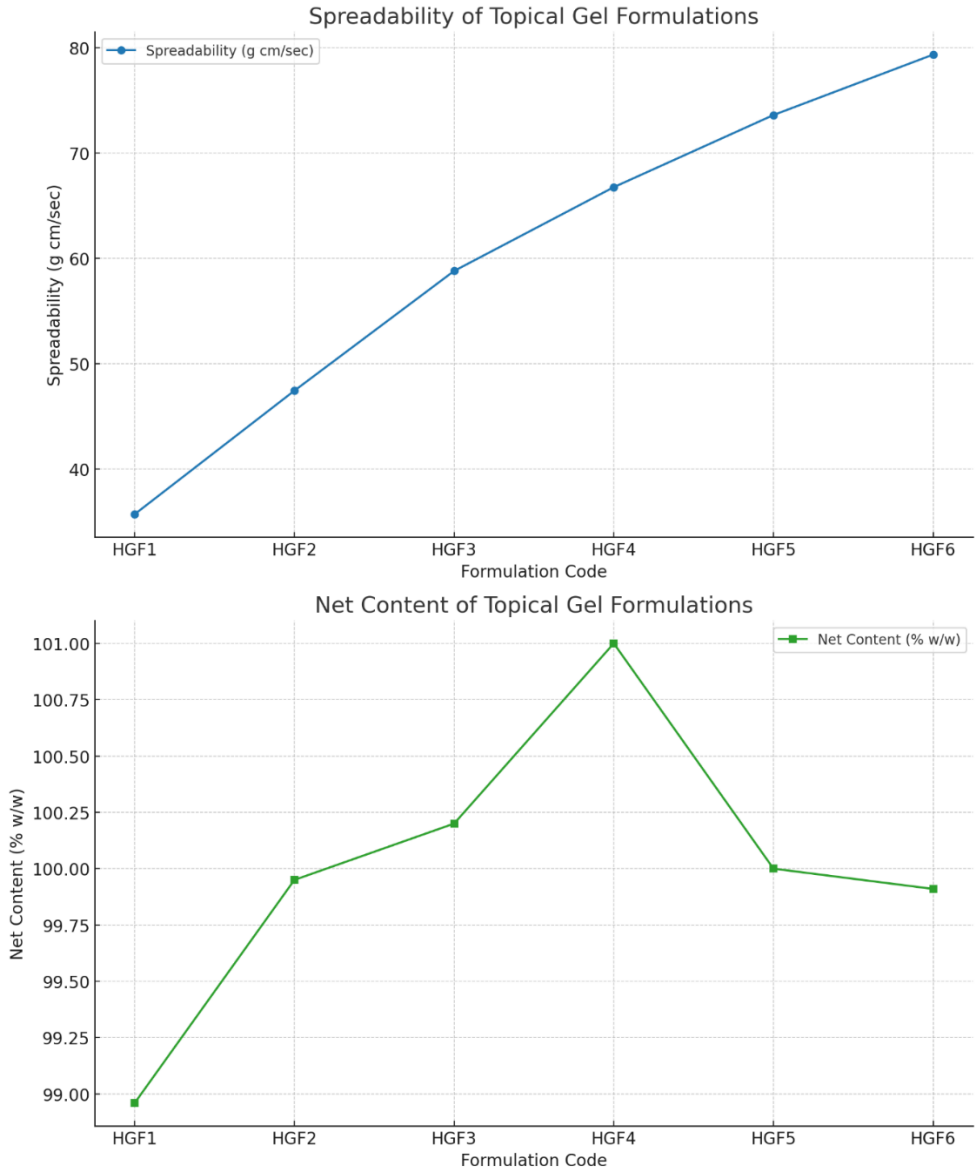

Figure 5. Spreadability and Net Content of Gel Formulations

Table 6. Extrudability and Physical Appearance for the topical gel formulations fabricated with Carbopol 934 at 1.5% concentration.

Code	Extrudability	Physical Appearance
HGF1	Excellent	Dark-greenish, homogeneous, and transparent
HGF2	Good	Dark-greenish, homogeneous, and transparent
HGF3	Good	Dark-greenish, homogeneous, and transparent
HGF4	Excellent	Dark-greenish, homogeneous, and transparent
HGF5	Excellent	Dark-greenish, homogeneous, and transparent
HGF6	Good	Dark-greenish, homogeneous, and transparent

3.6.6 Diffusion profile in vitro and kinetics of drug release

Table 7 presents the in vitro diffusion profile for six gels with variable compositions (HGF1 to HGF6), with a five-hour duration for cumulative drug release expressed as a percentage. Comparing each one, one can see how each releases its active compound over a period, which is relevant in terms of understanding its efficacy and potential application in a therapeutic scenario. HGF4 and HGF2 exhibit a significantly accelerated one-hour release of the drug, with values of 21.41 ± 1.20% and 21.28 ± 1.21%, respectively. That kind of accelerated

delivery could be beneficial in cases with a rapid onset of action demand. HGF1 and HGF5 have a slow and intermediate level of release, and that could be useful for use in cases in which an immediate increase in drug level is not desired. By three hours, HGF2 prevails with a high cumulative release of 73.38 ± 1.24%, followed closely by HGF4 and HGF6. Sustained diffusion could represent a less viscous preparation or one with high solubility of the active ingredient, facilitating quick absorption. By four hours, HGF2 reaches a high value of 93.37 ± 1.31%, then dips a little at five hours to 83.31 ± 1.29%. HGF4 reaches an unprecedented high reading of 102.42 ± 1.33% at five hours, which may be attributed to an experimental flaw, possibly over-saturation, resulting in an overshoot reading. HGF3, HGF5, and HGF6 build slowly and level off, eventually achieving a sustained release for long-term therapeutic activity. The overall diffusion profiles reveal that each gel possesses specific properties, making them applicable for a variety of therapeutic requirements. HGF2 and HGF4 are best suited for therapeutic requirements with a demand for rapid drug delivery, while HGF1, HGF3, HGF5, and HGF6 may be applicable for extended drug delivery, providing therapeutic efficacy over a prolonged period. Differences in the composition of the gel matrix probably cause these differences, with an impact on the kinetics of drug release and diffusion behaviour. This investigation is crucial for developing targeted topical drugs for specific medical conditions by manipulating these formational properties.

Table 7. Diffusion profile *in vitro*

TIME (Hr)	Gel Formulations					
	% Cumulative drug release					
	HGF1	**HGF2**	**HGF3**	**HGF4**	**HGF5**	**HGF6**
1	11.89 ± 1.20	21.41 ± 1.20	14.28 ± 1.19	21.28 ± 1.21	14.91 ± 1.20	17.39 ± 1.28
2	20.00 ± 1.19	38.01 ± 1.22	28.45 ± 1.20	37.17 ± 1.22	30.06 ± 1.20	31.00 ± 1.29
3	36.26 ± 1.22	73.38 ± 1.24	51.47 ± 1.26	56.34 ± 1.22	54.09 ± 1.27	58.99 ± 1.32
4	44.97 ± 1.23	93.37 ± 1.31	67.20 ± 1.29	73.27 ± 1.27	71.40 ± 1.28	69.08 ± 1.30
5	69.38 ± 1.30	83.31 ± 1.29	82.40 ± 1.30	102.42 ± 1.33	87.41 ± 1.29	86.90 ± 1.33

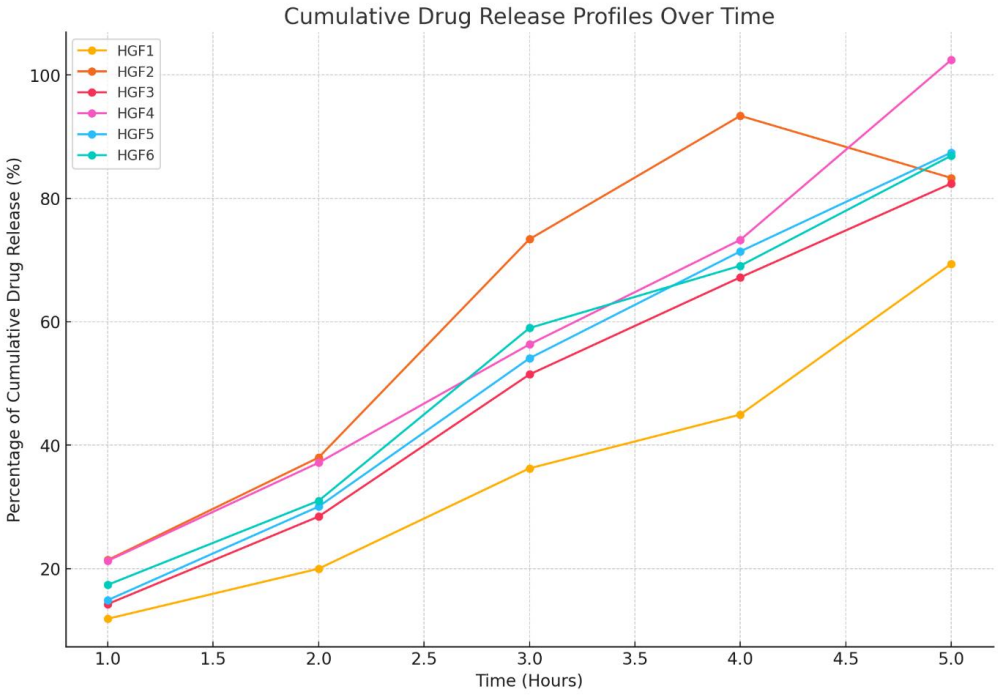

Figure 6. In vitro diffusion profile of topical herbal gels.

3.6.7 Kinetic modelling of data from in vitro releases

Table 8 presents a detailed examination of the kinetic modeling of six gel formulations (HGF1 through HGF6) under three kinetic models: Zero Order, First Order, and Higuchi Diffusion Model. For determining

the best-fitting model for each, a high value for R^2 is selected as the best-fit model for explaining the mechanism of drug delivery. The Zero Order model, which represents a constant release in terms of concentration, best fits HGF1, HGF3, HGF4, HGF5, and HGF6, with R^2 values of 0.9645, 0.9941, 0.9860, 0.9947, and 0.9816, respectively. That such a model holds for these drugs tells us that such drugs deliver at a constant level, a property beneficial in sustained level maintenance over a duration of time. High values for R^2 for such drugs confirm them to have a high probability of delivering drugs over a sustained duration, a property beneficial for long-term dosing needs. For HGF2, in contrast, the Higuchi model best describes it, with the delivery of the drug determined via diffusion in the gel matrix, yielding an R^2 value of 0.8876. That such a model best characterizes HGF2 informs us about its behavior, being most determined by the physico-mechanical properties of the gel, such as its porosity, compatibility with drugs, and its constituent materials. In general, kinetic modelling of such preparations not only helps describe their mechanism of delivery but also optimizes them for a desired therapeutic demand in terms of the desired profiles of delivery. All such information helps develop effective delivery systems capable of delivering a predictable and sustained therapeutic effect.

Table 8. Kinetic modelling of drug release data in vitro

Code for Formulations	Zero order	First Order	Higuchi diffusion model	Best-fitted model
	R^2	R^2	R^2	
HGF1	0.9645	0.8921	0.9214	Zero Order
HGF2	0.8468	0.7051	0.8876	Higuchi
HGF3	0.9941	0.9635	0.9843	Zero Order
HGF4	0.9860	0.9470	0.9524	Zero Order
HGF5	0.9947	0.9466	0.9843	Zero Order
HGF6	0.9816	0.9425	0.9764	Zero Order

3.7 The optimum formulation

HGF5 stands out with its exceedingly high R^2 value (0.9947) in Zero Order, indicating an exceedingly predictable and constant release profile. That will work ideally for therapies in which dosing accuracy is critical and in scenarios where variation in drug levels can threaten the efficacy of the therapy or patient safety. Thus, HGF5 can be considered an ideal delivery form for the long-term and sustained delivery of drugs, provided its physico-mechanical properties (viscosity, pH, etc.) and spreadability meet the requirements for therapeutic use. With such a form, one can hope for increased compliance, fewer side effects, and successful treatment for this disease.

4. Conclusions

The conclusion of this research work confirms the effectiveness of UDASP-HB in providing antioxidant and anti-inflammatory activity, supported by its high flavonoid level and its capacity to inhibit key anti-inflammatory enzymes. Optimal delivery performance was achieved for developed topical gel forms, characterized by sustained kinetics that support therapeutic activity. Most importantly, the herbal topical gel formulation, HGF5, exhibited the best release behavior, with implications for its application in long-term treatment. All observations validate UDASP-HB as a worthy therapeutic modality for the treatment of inflammatory diseases, providing a basis for future development in a clinical environment.

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