RESEARCH PAPER

Formulation And Evaluation Of An Anti-Inflammatory Herbal Gel Preparation Containing *Bidens Pilosa* l. Leaf Extract

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ABSTRACT

Objective: To formulate topical gels containing the ethanolic leaf extract of *Bidens pilosa* L. and to evaluate their physicochemical properties, in vitro release, skin safety, anti-inflammatory activity and stability.

Methods: The leaf extract was prepared by Soxhlet extraction with ethanol. Three gels (G1, G2 and G3) containing 5% w/w extract were prepared by the cold mechanical method using Carbopol-934 (1%), HPMC K4M (1%) or a combination of both as gelling agents. The gels were assessed for colour, homogeneity, pH, spreadability, viscosity and drug content (total phenolic content, Folin-Ciocalteu method). In vitro release was studied in a Franz diffusion cell over 8 h. Skin irritation was tested in albino rats, and anti-inflammatory activity was assessed by the carrageenan-induced rat paw edema model using a diclofenac gel as the standard. Stability was studied at 40 ± 2 °C and 75 ± 5% RH for 90 days.

Results: All gels were green, homogeneous and had a skin-compatible pH (6.9–7.1), with drug content above 94%. G3 (Carbopol-934 + HPMC K4M) gave the highest cumulative drug release (88.02% at 8 h) and showed no irritation. It reduced paw edema by up to 57.78% at 3 h ($p < 0.01$ vs. control), compared with 70.16% for the diclofenac gel. G3 remained stable for 3 months.

Conclusion: A gel containing *B. pilosa* leaf extract with a Carbopol-934/HPMC K4M base is a stable, non-irritant topical preparation with significant anti-inflammatory activity.

Keywords: *Bidens pilosa*, herbal gel, Carbopol-934, HPMC K4M, carrageenan-induced paw edema, anti-inflammatory, topical delivery

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INTRODUCTION

Topical drug delivery is increasingly favoured for both local and systemic therapy. The skin is easily accessible, has a large surface area with extensive exposure to the circulatory and lymphatic networks, and permits non-invasive administration. Among topical vehicles, gels are often preferred over ointments because they are non-sticky and require little energy to prepare [1]. A gel is a three-dimensional matrix of natural or synthetic polymer held in a hydrophilic liquid. After application the liquid evaporates, leaving the drug entrapped in a thin film of gel-forming matrix on the skin [2]. The rigidity of a gel arises from the network of interlocking gelling-agent particles, and the nature of those particles and their linkages determines the structure and properties of the gel [3].

Inflammation and the need for safer topical agents

Inflammation is the body's protective response to tissue injury, infection or irritation. It is marked clinically by redness, heat, swelling, pain and loss of function, and it

is driven by mediators such as prostaglandins, leukotrienes, histamine, nitric oxide and pro-inflammatory cytokines [13]. When the response is excessive or prolonged, it contributes to conditions such as arthritis, dermatitis, soft-tissue injury and chronic pain.

Non-steroidal anti-inflammatory drugs (NSAIDs) act mainly by inhibiting cyclooxygenase, while corticosteroids suppress several inflammatory pathways at once. Oral use of both groups can cause gastric irritation, renal and cardiovascular effects, and, with steroids, systemic and cutaneous adverse effects. Topical delivery reduces systemic exposure and places the drug close to the inflamed site, which is why topical NSAID gels are widely used for localised pain and swelling. The same logic motivates the search for plant-derived topical agents that are effective, well tolerated and affordable.

Herbal gels as topical vehicles

Plant extracts are complex mixtures, and their use on the skin requires a vehicle that releases the active constituents, spreads easily and is cosmetically acceptable. Hydrophilic gels suit this purpose well. They are water-washable, non-greasy and non-staining, they give a cooling effect on application, and they release water-soluble constituents such as phenolics and flavonoids readily. Carbopol-934, a cross-linked polyacrylic acid, forms clear, high-viscosity gels once neutralised but can feel tacky at high concentration. Hydroxypropyl methylcellulose (HPMC K4M) is a non-ionic cellulose ether that forms smooth, less viscous gels with good spreadability and film-forming ability. Combining the two may balance consistency, spreadability and release, and this was the working hypothesis of the present study.

Botanical profile of *Bidens pilosa* L.

Bidens pilosa L., a member of the Asteraceae (sunflower) family, is an annual herb that grows to roughly 0.3–1.5 m. It has opposite, pinnately divided leaves, small white-and-yellow flower heads, and barbed black achenes that cling to clothing and animals. The plant is a common weed in tropical and subtropical regions, including India, Africa, South America and Southeast Asia, and is known by local names such as Spanish needles, blackjack and beggar's ticks. In traditional medicine, leaf preparations are used as poultices and decoctions for wounds, boils, burns, skin infections, sore throat, gastrointestinal complaints, fever and inflammatory conditions [4].

Phytochemistry and reported pharmacology

The phytochemistry of *B. pilosa* has been reviewed extensively [4]. The main classes of compounds reported from the aerial parts are summarised in Table 1A. Flavonoids and phenolic acids are the groups most relevant to anti-inflammatory activity, and they are the constituents estimated as total phenolic content in the present study.

Table 1A. Constituent classes reported in *B. pilosa* and their relevance to inflammation [4]

Class	Examples reported	Relevance
Flavonoids	Quercetin and luteolin derivatives, okanin glycosides (e.g. marein), centaurein	Antioxidant; possible inhibition of cyclooxygenase, lipoxygenase and nitric oxide synthase
Phenolic acids	Caffeic, chlorogenic and ferulic acid derivatives	Radical scavenging; modulation of inflammatory signalling
Polyacetylenes	Phenylheptatriyne, cytopiloyne	Reported immunomodulatory and anti-inflammatory activity
Terpenoids and sterols	Various triterpenes and phytosterols	Possible contribution to membrane-stabilising and anti-edema effects

Beyond anti-inflammatory action, extracts of the plant have been reported to show antioxidant, antimicrobial, antimalarial, antidiabetic, hepatoprotective and wound-healing activity [4]. The combination of antioxidant and anti-inflammatory properties with traditional use on skin makes the leaf a logical candidate for a topical anti-inflammatory gel.

Rationale of the present work

Oxidative stress and inflammation are closely linked: reactive oxygen species activate inflammatory transcription factors, and inflammatory cells in turn generate more reactive species. An antioxidant-rich extract delivered in a topical gel may therefore act at several points in this cycle. Despite its traditional use, a systematically developed and evaluated gel of *B. pilosa* extract has not been widely reported.

Steroidal and non-steroidal anti-inflammatory drugs are associated with a wide range of adverse effects, which has directed research toward anti-inflammatory agents from natural sources. *Bidens pilosa* L. (Asteraceae) is a widely distributed herb used in traditional medicine for wounds, inflammation and various skin complaints [4]. Its leaves are rich in phenolic compounds and flavonoids and show strong antioxidant activity [4,5]. Flavonoids are known to act as antioxidants and as potential inhibitors of cyclooxygenase, lipoxygenase and nitric oxide synthase, which are key enzymes in the inflammatory cascade [6].

Although *B. pilosa* has long been used against inflammation, reports on gel formulations developed from its extract are scarce. This study therefore aimed to formulate herbal gels from the ethanolic leaf extract of *B. pilosa* using different gelling agents, and to evaluate their physicochemical characteristics, in vitro release, safety, anti-inflammatory activity and stability.

The specific objectives were:

- to prepare the ethanolic extract of *B. pilosa* leaves by Soxhlet extraction;
- to formulate three 5% w/w extract gels using Carbopol-934, HPMC K4M and their combination;
- to evaluate colour, homogeneity, pH, spreadability, viscosity and drug content of the gels;
- to study in vitro release of polyphenolic content through a diffusion membrane;
- to assess acute skin irritation and carrageenan-induced paw edema inhibition in rats against a diclofenac gel standard; and
- to test the stability of the best formulation under accelerated conditions.

MATERIALS AND METHODS

Materials and equipment

Carbopol-934, HPMC K4M, triethanolamine, methyl paraben, propyl paraben, ethanol, Folin-Ciocalteu reagent, sodium carbonate, λ-carrageenan and the buffer salts were of analytical or pharmaceutical grade. A commercial diclofenac gel (Voltaren) served as the standard. The principal equipment was a Soxhlet apparatus, rotary vacuum evaporator, mechanical stirrer, digital pH meter, Brookfield DV-II viscometer with helipath stand, UV-visible spectrophotometer, Franz diffusion cell assembly and digital plethysmometer.

Preparation of the ethanolic extract of *Bidens pilosa*

Bidens pilosa leaves were collected and authenticated by a qualified botanist [authentication details and voucher specimen number to be inserted]. The leaves were shade-dried and pulverised, then passed through a 40-mesh sieve to obtain a coarse powder. One hundred grams of powder was extracted with ethanol by hot extraction in a Soxhlet apparatus. The extract was cooled and filtered, and the filtrate was evaporated under vacuum to give a residue.

Preparation of the gel formulations

Three gels were prepared with the extract using Carbopol-934 alone (G1), HPMC K4M alone (G2), and a mixture of Carbopol-934 and HPMC K4M (G3), following the cold mechanical method of Schmolka [7]. Each polymer was weighed and dispersed in distilled water with continuous mechanical stirring, then left for 24 h at room temperature to hydrate fully. Methyl paraben and propyl paraben were added as preservatives with continuous stirring. Triethanolamine was added in small quantities to neutralise the gel to a skin-compatible pH. Finally, the extract was incorporated with stirring until completely dispersed. The gels were filled into aluminium collapsible tubes and sealed. A base gel without extract was prepared in the same way as the control.

Table 1. Composition of the gel formulations

Ingredient (% w/w)	G1	G2	G3
B. pilosa leaf extract	5	5	5
Carbopol-934	1.0	–	1.0
HPMC K4M	–	1.0	1.0
Triethanolamine	q.s.	q.s.	q.s.
Methyl paraben (0.5%)	1.5 mL	1.5 mL	1.5 mL
Propyl paraben (0.2%)	0.5 mL	0.5 mL	0.5 mL
Purified water to	100 mL	100 mL	100 mL

Table 1B. Function of each ingredient in the gel

Ingredient	Function
B. pilosa leaf extract	Active ingredient (anti-inflammatory, antioxidant)
Carbopol-934	Gelling agent; forms a high-viscosity network after neutralisation
HPMC K4M	Gelling agent; improves spreadability and film formation
Triethanolamine	Neutralising agent; raises pH so that Carbopol swells and thickens
Methyl paraben, propyl paraben	Preservatives against microbial growth
Purified water	Hydrophilic dispersion medium

The preparation sequence was as follows.

1. Weigh the polymer(s) and disperse in a measured quantity of distilled water under mechanical stirring.
2. Leave the dispersion for 24 h at room temperature for complete hydration.
3. Add methyl paraben and propyl paraben with continuous stirring.
4. Add triethanolamine dropwise until a neutral pH is reached and a clear gel forms.
5. Incorporate the extract (5% w/w) with stirring until it is uniformly dispersed.
6. Fill the gel into aluminium collapsible tubes and seal.
7. Prepare a placebo base in the same way without extract for use as the control.

Evaluation of the gel formulations

The gels were evaluated for colour, homogeneity, pH, spreadability, viscosity and drug content (total phenolic content).

pH. Five grams of gel was dispersed in 45 mL of water and the pH was read with a digital pH meter (Systronics 335, Ahmedabad). Each formulation was measured in triplicate and the mean was recorded [8].

Homogeneity. After the gels were set in their containers, they were inspected visually for appearance and for the presence of aggregates.

Viscosity. Viscosity was measured with a Brookfield DV-II viscometer using a helipath stand and a T-bar spindle (T95) [9]. Fifty grams of gel was placed in a 100 mL beaker, and viscosity was recorded at 2.5, 4, 5 and 10 rpm.

Spreadability. Spreadability was measured using a wooden block and glass slide apparatus fitted with a pulley [10], based on the slip and drag characteristics of the gel. About 2 g of gel was placed on the fixed ground slide and sandwiched with a second movable slide of equal size fitted with a hook. A 1 kg weight was placed on the slides for 5 min to expel air and form a uniform film. The top slide was then pulled with an 80 g weight through a string attached to the hook, and the time (s) taken for it to separate from the fixed slide was noted. A shorter time indicates better spreadability. Spreadability was calculated as:

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

where S is spreadability (g·cm/s), M is the weight tied to the upper slide, L is the length of the glass slide, and T is the time taken for the slides to separate.

Drug content. One gram of gel was accurately weighed and dissolved in phosphate buffer (pH 6.8). After suitable dilution, total phenolic content was determined by the Folin-Ciocalteu method with minor modification [11,18]. Absorbance was read at 765 nm with a UV-visible spectrophotometer, and the concentration was used to estimate drug content.

In vitro release of polyphenolic content

Release was studied in a Franz diffusion cell fitted with a dialysis membrane [10]. A 0.1 g sample was applied to the membrane in the donor compartment, and the membrane was kept in contact with the receptor compartment containing phosphate buffer (pH 6.8), stirred magnetically at 37 ± 1 °C. The study ran for 8 h, with samples withdrawn at 0.5, 1, 2, 4, 6 and 8 h and replaced with an equal volume of fresh buffer. Total phenolic content in each sample was determined by the Folin-Ciocalteu method at 765 nm.

Experimental animals

Animal experiments were carried out after approval by the Institutional Animal Ethics Committee [approval number to be inserted]. Albino Wistar rats of either sex weighing 150–200 g were housed at 22 ± 2 °C and 50 ± 5% humidity under a 12 h light/dark cycle, with a commercial stock diet and water *ad libitum*.

Acute skin irritation study

Rats (150–200 g) were divided into three groups of five. The dorsal hair was removed one day before the study, and the animals were housed individually to prevent contact with one another. One group served as control (plain gel base), one received the standard irritant (0.8% aqueous formalin), and one was the test group. Fifty milligrams of formulation was applied to a 1 cm² area of intact and abraded skin on different animals. The animals were observed for seven days for edema and erythema, which were assessed visually as described by Draize et al. [17].

Carrageenan-induced rat paw edema

Rats of either sex (150–200 g) were divided into control, standard and test groups of six animals each, with free access to food and water before the experiment. Test animals received 0.2 g of the *B. pilosa* gel (G3) rubbed gently onto the plantar surface of the right hind paw; the control group received the gel base only, and the standard group received diclofenac gel (Voltaren) in the same manner. One hour later, 50 µL of a freshly prepared 1% carrageenan suspension in saline was injected into the plantar surface of the right hind paw. Paw volume was measured with a plethysmometer immediately after the injection and at 1, 2, 3 and 4 h [12]. Percentage inhibition of edema was calculated as:

$$\% \text{ inhibition} = (Vc - Vt) / Vc \times 100$$

where Vc is the mean paw volume of the control group and Vt is the mean paw volume of the treated group.

Analysis of release kinetics

The cumulative drug release data were fitted to zero-order (Q against t), first-order (ln(100 − Q) against t)

and Higuchi (Q against √t) models by linear regression, and the coefficient of determination (R^2) was used to select the best-fitting model [14,15]. The Korsmeyer-Peppas model was not used because too few data points lay below 60% release.

Stability study

Stability of the best formulation (G3) was assessed according to the ICH guideline for accelerated conditions [16]. Gel in sealed aluminium tubes was stored at 40 ± 2 °C and 75 ± 5% relative humidity for 90 days. Samples were withdrawn at 0, 15, 30, 45, 60 and 90 days and examined for colour, viscosity and the percentage of drug content remaining.

Statistical analysis

Results are expressed as mean ± SEM. Data were analysed by one-way ANOVA followed by Dunnett's test using GraphPad InStat version 3.00. A probability of $p < 0.05$ or less was considered statistically significant.

RESULTS AND DISCUSSION

Formulation and physicochemical evaluation

Carbopol-934 and HPMC K4M were used as gelling agents, triethanolamine to neutralise the pH, and methyl and propyl paraben as preservatives. All gels were green with an aromatic odour, good homogeneity and good spreadability. The pH of the gels ranged from 6.9 to 7.1, which lies within the normal pH range of skin, so irritation on application is unlikely. The gel with Carbopol-934 alone (G1) was the most viscous and the least spreadable, whereas the HPMC K4M gel (G2) was the least viscous and the most spreadable. The combination gel (G3) fell between the two. Drug content exceeded 94% in all formulations and was highest in G3 (97.2%).

Table 2. Physicochemical evaluation of the *B. pilosa* gels

Formulation	Colour	Homogeneity	pH	Spreadability (g·cm/s)	Drug content (%)
G1	Green	Good	6.9 ± 0.04	20.30 ± 1.11	94.6 ± 0.3
G2	Green	Good	7.1 ± 0.01	50.05 ± 1.06	95.9 ± 0.1
G3	Green	Good	7.0 ± 0.01	31.25 ± 1.51	97.2 ± 0.2

Values are mean ± SD, n = 3.

Table 3. Viscosity of the *B. pilosa* gels

Formulation	2.5 rpm	4 rpm	5 rpm	10 rpm
G1	20108 ± 20	15300 ± 11	10014 ± 16	8600 ± 18
G2	2345 ± 10	2100 ± 12	1980 ± 12	1425 ± 15
G3	15163 ± 11	10986 ± 18	9030 ± 10	7800 ± 13

Viscosity in cps. In all gels viscosity fell as the rpm rose, showing shear-thinning (pseudoplastic) flow, which is desirable for topical products.

Interpretation of formulation behaviour

The three gels differ mainly in consistency, and consistency governs how the product feels, spreads and releases its contents. G1, with Carbopol-934 alone, had the highest viscosity (20108 cps at 2.5 rpm) and the lowest spreadability (20.30 g·cm/s). Carbopol forms a tightly cross-linked, ionised network after neutralisation, which gives a thick gel that resists flow and is harder to spread over a large area. G2, with HPMC K4M alone, was about eight to nine times less viscous (2345 cps) and spread most easily (50.05 g·cm/s), but such a thin gel may run off the skin and offers less retention at the site of application.

G3 combined both polymers and gave intermediate values for viscosity (15163 cps) and spreadability (31.25 g·cm/s). Its viscosity was close to that of G1, but its spreadability was about 50% higher. This suggests that HPMC interrupts the dense Carbopol network enough to ease spreading without making the gel too thin. A product that spreads easily needs less force and gives more uniform coverage, which improves dose uniformity and patient acceptance.

The pH of all three gels (6.9–7.1) is slightly above the usual skin surface pH of about 4.5–6, but it lies in the range that is normally tolerated and is needed for Carbopol to swell fully. The drug content (94.6–97.2%) shows that the extract was incorporated with little loss, and that the polyphenols were not degraded by neutralisation or by 24 h of polymer hydration. The small standard deviations indicate good batch reproducibility.

In vitro release

Release of polyphenolic content over 8 h is shown in Table 4. G3 released the most phenolic content at 8 h (88.02%), compared with G1 (70.05%) and G2 (75.13%). The gel combining Carbopol-934 and HPMC K4M therefore released the active constituents better than the single-polymer gels. Average flux was highest during the first hour for all formulations and then decreased gradually with time, and flux values were generally greater for G3.

Table 4. In vitro release of polyphenolic content

Time (min)	G1 %CDR	G1 flux	G2 %CDR	G2 flux	G3 %CDR	G3 flux
30	1.10 ± 0.01	0.60	2.01 ± 0.03	1.09	4.00 ± 0.05	2.17
60	10.50 ± 0.05	5.11	18.05 ± 0.07	8.72	29.01 ± 0.07	13.59
120	21.13 ± 0.09	2.89	30.06 ± 0.06	3.26	49.65 ± 0.02	5.61
240	48.69 ± 0.02	3.75	56.09 ± 0.03	3.54	79.31 ± 0.06	4.03
360	59.12 ± 0.06	1.42	68.13 ± 0.05	1.64	84.85 ± 0.01	0.75
480	70.05 ± 0.06	1.49	75.13 ± 0.03	0.95	88.02 ± 0.06	0.43

%CDR = cumulative drug release; flux in µg/cm²/min. Values are mean ± SD, n = 3.

Release kinetics

Fitting the release data to three models (Table 4A) showed that first-order kinetics described all three gels best, followed closely by the Higuchi model. This pattern is typical of a water-soluble constituent diffusing from a hydrophilic matrix, in which the release rate falls as the concentration gradient declines. Zero-order release fitted poorly, particularly for G3, because its release flattened after 4 h as most of the phenolic content had already left the gel.

Table 4A. Release kinetic parameters (derived from the data in Table 4)

Formulation	Zero-order R^2	First-order R^2	Higuchi R^2	First-order k (min^{-1})	Higuchi k (% $min^{-½}$)
G1	0.959	0.993	0.991	0.0027	4.33
G2	0.927	0.986	0.984	0.0031	4.50
G3	0.818	0.940	0.921	0.0047	5.09

The rate constants rank the gels G3 > G2 > G1 under both the first-order and Higuchi models, which agrees with the cumulative release and flux results. G3 released phenolics roughly 1.8 times faster than G1 by the first-order constant. The faster release from G3 despite its high viscosity is of interest. A possible explanation is that the mixed Carbopol/HPMC network has a more open structure and holds water more freely than the Carbopol-only network, so that the phenolic constituents diffuse out more easily. This interpretation is a hypothesis and would need confirmation by rheological or microstructural studies. Because the kinetic fits rest on six time points, they should be read as descriptive rather than definitive.

Acute skin irritation

No edema or erythema was seen in any animal during the seven days after application, indicating that the *B. pilosa* gels did not cause skin irritation or toxicity on topical use.

Skin safety is a basic requirement for any topical product, because a gel that irritates would worsen the inflammation it is meant to treat. The absence of reaction on both intact and abraded skin is encouraging, since abraded skin is more permeable and more sensitive. It suggests that the extract, the polymers and the preservatives are well tolerated at the concentrations used, whereas the formalin group, as the positive control, is expected to show a reaction. The test covered a single application and a small number of animals, so repeated-dose and human patch tests would still be needed.

Anti-inflammatory activity

The anti-inflammatory activity of the selected formulation (G3) is shown in Table 5.

Table 5. Paw volume (mL) in carrageenan-induced paw edema in rats (percentage inhibition in brackets)

Treatment	1 h	2 h	3 h	4 h
Control (gel base)	1.21 ± 0.02	1.20 ± 0.17	1.22 ± 0.02	1.09 ± 0.03
Diclofenac gel	0.70 ± 0.07** (42.07)	0.52 ± 0.02** (56.16)	0.36 ± 0.03** (70.16)	0.26 ± 0.08** (75.00)
G3	0.91 ± 0.04** (24.75)	0.75 ± 0.02** (37.50)	0.51 ± 0.04** (57.78)	0.60 ± 0.02** (45.35)

Values are mean ± SEM, n = 6; one-way ANOVA followed by Dunnett's test. **$p < 0.01$ vs. control.

G3 reduced paw edema by 57.78% at 3 h after carrageenan injection compared with the gel base, and significantly reduced paw volume throughout the observation period ($p < 0.01$). Its effect was smaller than that of the diclofenac gel but clearly significant. The G3 gel, containing 1% Carbopol-934 and 1% HPMC K4M, was therefore judged the best formulation for topical anti-inflammatory use.

The activity is probably due to the polyphenols, mainly flavonoids, in the *B. pilosa* extract, which act as antioxidants and may inhibit cyclooxygenase, lipoxygenase and nitric oxide synthase [6]. *B. pilosa* is used traditionally on the skin for wounds, boils, burns and eczema, and the plant is reported to relieve pain and swelling [4]. A gel made from the extract can thus provide a suitable topical form that combines these benefits with anti-inflammatory action.

Comparison with the standard drug

The diclofenac gel gave greater inhibition than G3 at every time point, rising from 42.07% at 1 h to 75.00% at 4 h. G3 reached 24.75% at 1 h and a maximum of 57.78% at 3 h, which is about 82% of the effect of the standard at that time. At 4 h the effect of G3 fell to 45.35% while that of diclofenac continued to rise, suggesting that G3 acts for a shorter period, so a repeat application might be needed. This is expected for a crude extract tested against a purified, potent drug, and a 5% crude extract that achieves more than half the standard's effect at its peak is a promising result. Both treatments differed significantly from the control ($p < 0.01$).

Possible mechanisms of action

Carrageenan-induced edema is biphasic. The early phase (about the first 1.5 h) is linked to histamine and serotonin release, and the later phase (from about 1.5 to 6 h) is linked to prostaglandins, bradykinin and nitric oxide produced by cyclooxygenase and nitric oxide synthase. The increasing inhibition shown by G3 from 1 h to 3 h is compatible with an effect on the later, prostaglandin-mediated phase. The following mechanisms may contribute, although none was tested directly in this work:

- inhibition of cyclooxygenase and lipoxygenase by flavonoids, which reduces prostaglandin and leukotriene synthesis [6];
- inhibition of nitric oxide synthase, which lowers nitric oxide-mediated vasodilation and edema;
- scavenging of reactive oxygen species, which limits oxidative amplification of inflammation; and
- membrane-stabilising and anti-exudative effects of other constituents such as terpenoids and polyacetylenes [4].

The gel base itself may also add a mild cooling and hydrating effect, but this was controlled for by the use of a base-only group.

Stability study

Table 6. Stability of gel G3 at 40 ± 2 °C and 75 ± 5% RH

Time (days)	Colour	Viscosity (cps)	Drug remaining (%)
0	Green	15163 ± 11	97.23 ± 0.2
15	Green	15287 ± 09	97.05 ± 0.4
30	Green	15890 ± 12	96.86 ± 0.1
45	Green	16200 ± 09	96.05 ± 0.3
60	Green	16478 ± 10	95.93 ± 0.2
90	Green	16780 ± 09	95.01 ± 0.2

Colour was unchanged, viscosity changed only slightly and about 95% of the drug content remained after 90 days, indicating that the G3 gel is stable for at least 3 months under accelerated conditions.

The small rise in viscosity over 90 days is commonly seen when a gel loses a little water at 40 °C, and the gradual fall in drug content (about 2.2 percentage points) is consistent with slow oxidation of phenolic constituents. Both changes are small enough to be acceptable. Real-time studies at 25 ± 2 °C should still be run to establish shelf life.

Overall appraisal of the formulations

Table 7. Comparative summary of the three gels

Parameter	G1 (Carbopol)	G2 (HPMC)	G3 (Carbopol + HPMC)
Viscosity at 2.5 rpm (cps)	20108	2345	15163
Spreadability (g·cm/s)	20.30	50.05	31.25
Drug content (%)	94.6	95.9	97.2

Parameter	G1 (Carbopol)	G2 (HPMC)	G3 (Carbopol + HPMC)
Release at 8 h (%)	70.05	75.13	88.02
First-order k (min^{-1})	0.0027	0.0031	0.0047
Overall	Thick, hard to spread	Easy to spread, very thin	Best balance and release

G3 had the highest drug content and drug release and a workable balance of viscosity and spreadability, so it was chosen for the biological and stability studies. G1 and G2 were not tested in vivo, so their anti-inflammatory activity remains unknown.

LIMITATIONS AND FUTURE SCOPE

This study has several limitations. Only one extract concentration (5% w/w) was used, so no dose-response relationship was established. Total phenolic content is a general marker and does not identify the individual compounds responsible for the activity. Only G3 was tested for anti-inflammatory activity, and the carrageenan model reflects acute inflammation only. The skin irritation test was observational and did not use a scored scale, and the stability study was run for 90 days under accelerated conditions only.

Future work should address the following points:

- standardise the extract by HPLC fingerprinting and quantify marker compounds such as quercetin, luteolin and chlorogenic acid;
- test several extract concentrations and add a gel-base plus extract dose-response arm;
- study chronic and other models, such as cotton-pellet granuloma, UV-induced erythema or wound healing;
- measure inflammatory markers (prostaglandin E2, cytokines, nitric oxide) in paw tissue;
- carry out ex vivo skin permeation and retention studies, and assess rheology and microbial limits;
- conduct real-time stability studies and a dermal safety study before any human trial.

If these studies confirm the present findings, a standardised *B. pilosa* gel could be developed as a low-cost herbal alternative for mild to moderate local inflammation.

CONCLUSION

Stable herbal gels containing 5% *Bidens pilosa* leaf extract can be prepared using Carbopol-934 and HPMC K4M. The gel combining both polymers (G3) showed the best drug content and release, caused no skin irritation, and significantly reduced carrageenan-induced paw edema in rats. These findings support the traditional topical use of *B. pilosa* and suggest the G3 gel as a promising, safe herbal alternative for managing inflammation, subject to further phytochemical standardisation and clinical evaluation.

The work shows that a simple, low-cost process (Soxhlet extraction followed by cold mechanical gelling) can turn a widely available medicinal weed into a pharmaceutically acceptable topical product. The choice of gelling agent had a clear effect on the quality of the gel, and the mixed Carbopol-934/HPMC K4M base gave the best compromise of consistency, spreadability, and release.

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CONFLICT OF INTERESTS

Declared none.

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