



Formulating Topical Gel Product containing *Dolichos biflorus* Standardized Seeds Extract and *Cichorium intybus* Standardized Roots Extract for treating Inflammation-related Skin Ailments

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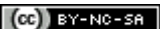
ABSTRACT

Both *Dolichos biflorus* and *Cichorium intybus* have demonstrated noteworthy anti-inflammatory activity in animal models, on individual capacity. Recently, the combined synergistic *in vitro* anti-inflammatory activity of *D. biflorus* and *C. intybus* has been reported which opened new avenues of research. Inspired by this, we decided to produce a topical gel formulation that will have perspectives in treating various skin ailments. The present research aimed at developing two topical gel formulations (F1 and F2) utilizing the components such as Carbopol 934, methylparaben, propylparaben, propylene glycol 400, and triethanolamine. The products were suitably evaluated by employing the following recommended tests: visual clarity, transparency, solubility, pH, washability, removal, viscosity, homogeneity, spreadability, extrudability, thermal stability, density, freeze-thaw cycle, centrifugation test, non-volatile matter, swelling index, irritation test, and accelerated stability. The study concluded that both the plant extracts (*D. biflorus* and *C. intybus*) with prominent anti-inflammatory activities were successfully incorporated into the Carbopol-based topical gel formulations. As a result, it was concluded that the formulation may be a very effective solution for the treatment of inflammatory diseases on a topical or transdermal basis. However, additional preclinical, clinical, and long-term stability research is required.

Keywords: *Dolichos biflorus*, *Cichorium intybus*, Anti-inflammatory, Topical Gel, Formulations, Evaluation

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INTRODUCTION

Inflammation of the skin is a symbol of the body's immune reaction. Redness, sweat, scratching, sensitivity, and swelling are all possible symptoms. Skin inflammation may be acute, such as from a skin infection, or persistent, such as from an inflammatory disease like psoriasis.^[1] The majority of cases of skin inflammation are treatable, although the cure is determined by the cause of the inflammation. When bacteria or other foreign objects penetrate the skin from a cut or wound, skin infections arise. Skin diseases are more likely in people who have weakened immune systems.^[2] Diabetes, low breathing, advanced age, and obesity are also risk factors. Although certain bacteria just damage a specific area of skin, others may penetrate further through the layers of the skin and even beyond. Bacterial skin infections entail cellulitis, impetigo, and staphylococcal infections, which are triggered by bacteria invading the skin.^[3] Viruses induce viral diseases, which involve shingles and warts. Athlete's foot and yeast infections are examples of fungal infections triggered by fungus invading the skin. Finally, viruses such as lice and scabies induce bacterial skin infections.^[4]

Dolichos biflorus L. (Family: Leguminosae) is a valuable medicinal plant used in Ayurveda and Unani medicine, especially for the removal of kidney stones. The seeds are used as an astringent, diuretic, and tonic, as well as an anti-diaphoretic in powder form. Hemorrhoids, cancer, bronchitis, heart failure, colic, worms, flu, urticaria, and rheumatoid arthritis are also treated for them. Seed decoction is used to treat leucorrhoea and menstrual conditions, and it even serves as an anthelmintic when combined with milk. Pulse soup is a good diet to follow if you have a subacute case of swollen liver and spleen.^[5]

The roots of *Cichorium intybus* L. (Asteraceae) have long been used in Ayurvedic medicine. Gallstones, gastroenteritis, sinus infections, asthma, and constipation are among the conditions for which it is prescribed. It's a coffee alternative, and its leaves, flowers, nuts, and roots have been used as natural remedies for centuries. Chicory was once used as a medicinal herb, a coffee substitute, and a vegetable crop by ancient Egyptians, and it was even used as animal fodder on occasion. The root of chicory was discovered to produce up to 40% inulin in the 1970s, which has a significant impact on glucose and is also ideal for diabetics.^[6]

Both *D. biflorus* and *C. intybus* have demonstrated noteworthy anti-inflammatory activity in animal models, on individual capacity.^[7-10] Recently, the combined synergistic *in vitro* anti-inflammatory

activity of *D. biflorus* and *C. intybus* has been reported which opened new avenues of research. Inspired by this, we decided to produce a topical gel formulation that will have perspectives in treating various skin ailments.^[11]

The present research aimed at developing two topical gel formulations (F1 and F2) utilizing the components such as Carbopol 934, methylparaben, propylparaben, propylene glycol 400, and triethanolamine. The products were suitably evaluated by employing the following recommended tests: visual clarity, transparency, solubility, pH, washability, removal, viscosity, homogeneity, spreadability, extrudability, thermal stability, density, freeze-thaw cycle, centrifugation test, non-volatile matter, swelling index, irritation test, and accelerated stability.

MATERIALS AND METHODS

Chemicals: The reagents, consumables, and chemicals for this study were purchased via a local distributor from HiMedia[®] India Pvt. Ltd., Mumbai. S.A. Herbal Bioactive, Mumbai, Maharashtra provided standardized (NLT 45% Saponins) *D. biflorus* seed extract, and Green Heavens Private Limited, Nagpur, Maharashtra provided standardized (Bitters NLT 1.5% / 10:1) *C. intybus* root extract.

Instruments: The water used for the experiment was procured from a double-distilled water apparatus (Borosil[®], India). The Accro Tech[®] electronic balance (Model: AT-266-1, India) was used for measuring the chemicals. The pH of solutions was measured using a digital pH meter (Contech[®]). Brookfield[®] viscometer (DV-III programmable Rheometer) was employed to measure the viscosity. The centrifugation was performed with CentriBio[®] 80-2B equipment. The conductivity was measured through a Labtronics[®] digital conductivity meter (Model: ABS-1, India), Stability chamber (Bio-Technics[®], India) was utilized for accelerated stability studies.

Preparation of Formulations: 1 g of Carbopol 934 was dispersed in 50 mL of distilled water and the beaker was kept aside to swell the content for half an hour. Further, stirring was done to mix the Carbopol 934 to produce a gel consistency. 5 mL of distilled water was taken and the required quantities of preservatives (methylparaben and propylparaben) were dissolved by heating on a water bath. The solution was cooled and propylene glycol 400 was added. Further, the required quantities of standardized *D. biflorus* seed extract and standardized *C. intybus* root extract were mixed to the above mixture and volume made up to 100 mL by adding remaining distilled water.

Penultimately, the full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring. Finally, triethanolamine was added drop-wise to the formulation for adjusting

the required skin pH (6.8-7.2) and to obtain the gel at the required consistency. **Table 1** indicates the ingredients used for formulating this herbal topical gel product.

Table 1. Topical herbal gel formulations detail.

INGREDIENTS	FORMULATION-1 (F1)	FORMULATION-2 (F2)
<i>Dolichos biflorus</i> Standardized Seeds Extract	1 g	0.5 g
<i>Cichorium intybus</i> Standardized Roots Extract	0.5 g	1 g
Carbopol 934	1 g	1 g
Methylparaben (0.5%)	0.2 mL	0.2 mL
Propylparaben (0.2%)	0.1 mL	0.1 mL
Propylene glycol 400 (5%)	5 mL	5 mL
Triethanolamine	1.2 mL	1.2 mL
Distilled water	q.s.	q.s.

Evaluation

The evaluations of the fabricated formulations were performed as per the methods given by Mahajan *et al.*, 2017^[12]; Shivhare *et al.*, 2019^[13]; Borkar *et al.*, 2020^[14]; and Mahajan *et al.*, 2017a^[15].

Visual clarity: The prepared formulations were visually tested for clarity, appearance, color, and consistency against a black and white background.

Transparency: 5 mL of formulated gel was taken in the 10 mL test tube and its transparency was visually determined.

Stickiness: The stickiness was determined by adding a limited amount of gel and testing for the existence or absence of stickiness after the formulation was applied.

Solubility: The solubility of gel was estimated by using diverse solvents like water, methanol, propylene glycol, glycerin, acetone, and petroleum ether at room temperature.

pH: The pH of the dermal gel was measured using an automated pH meter that was calibrated. The glass electrode was dipped in 1 g of the formulation dissolved in 25 mL of distilled water before a constant reading was achieved. For each formulation, the pH was measured three times and the average was reported.

Conductivity: The glass electrode was optimized using the equipment's solutions (pH of 4.00 and 7.00), and the conductivity was measured in millivolts (mV). When weighing, the gel preparation was left for around 15 mins to achieve equilibrium. The conductivity of the product was tested three times and the average values were determined.

Spreadability: The spreadability of the polyherbal dermal gel was calculated using the concept of the slip-drag function. The protocol consisted of inserting 2 g of formulation on a ground slide and sandwiching it between equivalent glide slides with a hook attached. To clear the entrapped air and form a standardized film between the slides, a heavy mass was added to the slides. The residual gel material was scraped off from the edges. The top slide was then made to drag with a 50 g pressure. The time needed by the top slide to cover a distance of 6 cm was determined from the formula:

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

Where, S = Spreadability, M= Weight in the pan (tied to the upper slide) L = Length moved by the glass slide T = Time (in sec) taken to separate the slide completely each other.

Washability: The washability of formulations was tested by first applying the gel to the skin and then assessing the ease and degree of washing it with distilled water while manually examining the result.

Removal: The easiness of removal of the applied gel formulations was examined by applying the gel on the skin and then rubbing the applied part with tissue paper.

Extrudability: Filling 100 g of gel into capped collapsible aluminum tubes and sealing them with a manual ointment sealing system was used to test the extrudability of the prepared formulation. The tubes (which included various formulations) were sandwiched between two slides and securely clamped. After that, a 500 g weight was placed on the slides, and then the cap was opened, and the extruded ribbon length was measured after 10 mins.

Viscosity: The viscosity of the gel product was measured using the Digital Brookfield Viscometer at a temperature of $25\pm 1^\circ\text{C}$ and spindle no. 6 at 10 rpm. An adequate amount of gel was filled in a suitable big mouth jar in such a way that it would cause the spindle to be dipped and left to settle for 30 mins before the measurements.

Homogeneity: After the gel had settled in appropriate beakers, all of the prepared formulations were visually inspected for homogeneity (appearance, presence of any aggregates, kind of stain, after-feel, and removal of gel).

Thermal stability: The gel was inserted into a glass container with the aid of a spatula and tapped to stabilize at the rim. The bottle was filled to two-thirds capacity, a plug was placed, and the cap was tightened. The physical appearance of this filled container was registered after it was held upright in the incubator at 45°C for 48 hrs.

Loss on Drying: It is measured using 1 g of the formulation dried in a 105°C oven for 3 hrs. To analyze chemicals, combine them, and measure them precisely. The samples were placed in a container, the lid was closed, and the bottle and contents correctly by soft, sidewise shaking was measured. The samples were spread as equally as possible to a depth of around 5 mm, then placed the filled bottle in the drying chamber and the samples were dried at the specified temperature for constant weight. Before measuring, immediately the chamber was closed and enables the container to come to room temperature in desiccators. Loss on drying is calculated by the formula:

$$\% \text{ LOD} = (W_2 - W_3) \times 100 / (W_2 - W_1)$$

Where, W_1 = Weight of empty weighing bottle, W_2 = Weight of weighing bottle + Sample, W_3 = Weight of weighing bottle + Dried sample

Density: The pycnometer, which is a glass flask with a close-fitting glass stopper with a capillary hole in it, was used to determine the density of the formulations. A pycnometer was adjusted by weighing it before filling it with each gel formulation and weighing it again after filtering out any excess gel. The density of gel was calculated:

$$\rho = m / v$$

Where ρ is the density (g/mL), m is the mass of gel alone (g), and v is the volume of pycnometer (mL)

Freeze-Thaw Cycle: The gel sample was subjected to a freeze-thaw period, which took 12 days and six cycles to complete. The material was held at a certain temperature for 24 hrs throughout each series. The refrigerator temperature was $5\pm 2^\circ\text{C}$, and the greenhouse temperature was $40\pm 2^\circ\text{C}$.

Centrifugation test: In a tapered test tube, 10 g of the formulation was applied, and the sample gel was centrifuged at 3000 rpm for 30 mins at room temperature.

Swelling index: 2 g of the prepared dermal herbal gel was placed in a beaker comprising 10 mL of distilled water to assess the swelling index. The swelled formulation was withdrawn from the beaker and placed on a Petri dish after 1 hr. The content was re-weighed and the swelling index was estimated from the formula:

$$\text{Swelling index (Si)} = (W_t - W_o) / W_o \times 100$$

Where, W_t = weight of swollen at t time; W_o = original weight of gel at zero time.

Irritation test: The prepared gel was spread in a quantity of 0.5 g to regular hairless skin over a 6 cm^2 region and then wrapped with a semi-occlusive bandage for 1 hour. The bandage was withdrawn after the treatment period had passed, the applied gel was scraped off entirely, and the region was visually examined for any rashes or other signs. The test was carried out over 7-day duration. Grades were used to express the findings.

Non-Volatile Matter: In an evaporating dish, 1-5 g of the prepared gel was weighted and heated on a steam bath before much of the volatile matter had escaped. The weight was taken after 2 hrs of heating at 105°C in an oven, cooling in desiccators. The heating, cooling, and measuring process was repeated until the mass differential between two consecutive weights did not surpass 1 mg. The formula for calculation includes:

$$\text{Non-Volatile Matter} = (m_2 - m_3) \times 100 / (m_1 - m_3)$$

Where, m_1 = mass (in g) of the dish with the sample; m_2 = mass (in g) of the dish after heating; and m_3 = mass (in g) of the empty dish.

Accelerated Stability Study: In a stability chamber, the formulations were subjected to an accelerated stability analysis ($40^\circ\text{C}\pm 2^\circ\text{C}$ temperature; $75\% \pm 5\%$ relative humidity) for 30 days. The prepared gel solution was stored in a polyvinyl chloride jar with a black foil covering. The critical parameters have been determined.

Statistical analysis: The results were given as mean \pm standard deviation after experimenting three times and statistically analyzed using a t -test, $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Evaluation of Gel Formulations

Visual clarity: The two fabricated gel formulations (F1-F2) displayed a pale brown, less translucent, hazily transparent, and homogenous smooth

textured appearance with no solid particles or grittiness. Overall, the formulations have desired organoleptic properties with elegant looks and can be acceptable for the patients (or high patient compliance).

Transparency: The formulations were not at all transparent, rather the developed products have a low level of translucency. This translucency characteristic is largely influenced by the concentration of carbopol 940 in the gel formulations (F1-F2). A lower % of carbopol 940 leads to an enhancement in the clarity of the gel formulations.

Homogeneity: On touching between the fingers, no solid particles or grittiness have been found in both the formulations (F1-F2) which proved a high degree of homogeneity. A very high degree of homogeneity indicated the excellencies in the quality attributes and eventually their mass acceptability.

Stickiness: The results clearly suggested that the formulated gels (F1-F2) were free from stickiness after application, and they spread freely on the skin. These attributes conclude the easy application over the skin surface, no such stickiness with clothes, easy removal, and ultimately better patient compliance and acceptability.

Removal: The study revealed that both the topical herbal gel formulations (F1-F2) can be easily removed by tissue paper without any marking on the skin. This feature enables accidental removal of the formulations by the patients, in the conditions of any irritation or his/her own desirability.

Washability: A brilliant washability attribute has been observed for all the developed formulations (F1-F2). This feature enables the patient to easily wash off the formulations of his/her own will from the area of the application under accidental conditions. This enhances the patient's compliance and acceptability for the developed herbal topical product.

Non-volatile component: The non-volatile component (like terpenes and other small compounds) was found to be 12.78% w/w and 11.93% w/w for Formulation-1 and Formulation-2, respectively. It can be concluded that the volatile components (like ellagitannins, steroids, saponins, flavonoids, alkaloids, and phenolic resins) for the Formulation-1 and Formulation-2 have been identified as 87.22% w/w and 88.07% w/w, respectively which escaped from the product on heating at 105°C temperature for 2 hrs duration.

Centrifugation test: The centrifugation test revealed no noticeable instability in both the developed formulations (F1-F2). The study concluded that the gel formulations are profoundly stable and will not demonstrate any physical characteristics defects such as phase separation or frothing.

Thermal test: Both the formulations (F1-F2) passed the thermal test which confirmed their stability under heat stress. No phase separation, breaking, cracking, migration, or frothing was observed in both the gel formulations. However, it is strongly intended that the topical gel products must be stored at a cool, dry place even though they demonstrate stable nature at high temperatures for a long duration.

Solubility: In the solubility analysis, both the gel formulations (F1-F2) were practically insoluble in distilled water, partially soluble in methanol, and completely soluble in propylene glycol, glycerin, acetone, and petroleum ether. This concludes that the gel formulation contains abundant non-polar components as a result of formulation ingredients and herbal components (especially phytoconstituents), while the contents of polar components are quite negligible.

Loss on drying: The loss on drying was less than the specified limits (not more than 0.5%). The loss on drying is $0.22 \pm 0.06\%$ for Formulation-1 and $0.13 \pm 0.04\%$ for Formulation-2. This parameter concluded that the formulations have enough moisture / humidity content that is intended to form a gel film as well as to provide a humid environment to the inflamed area.

Irritation test: On the application of the gel formulations (F1-F2) for 7 days, no skin irritation, edema, rashes, erythema, or any dermatological reaction or specific inflammation were observed. This concludes that the formulation is extremely safe for topical applications.

pH: The pH of the gel formulations was found to be 7.3 ± 0.2 and 7.1 ± 0.1 for Formulation-1 and Formulation-2, respectively, which lies in the normal pH range of the skin. This parameter concludes that the formulations will not produce any irritation, pain, or discomfort to the patients.

Conductivity: The conductivity was found to be 66.41 ± 5.65 mV for Formulation-1 and 51.22 ± 4.35 mV for Formulation-2, respectively. A low to moderate conductivity has been observed which concluded that the gel can only be applied to the topical areas and is not intended for any specific ultrasound transmission applications, or in other words for internal applications.

Viscosity: Viscosity is an imperative factor that influences pharmaceutical properties such as spreadability, extrudability, pourability attribute from the container, etc. The viscosity of the formulations lies in the range of 41800 ± 600 cps for Formulation-1 and 38200 ± 900 cps for Formulation-2. The rheological study indicated that with an increase in the torque, the shear stress extensively increases which results in a decrease in the formulation viscosity.

Density: The measurement of density using a pycnometer was carried out for both formulations. Filling the pycnometer was so difficult because of the high viscosity of the formulations. The results showed that the densities of these formulations were near the density of water (1.173 ± 0.27 g/cm³ for Formulation-1 and 1.126 ± 0.43 g/cm³ for Formulation-2) as a result of high aqueous content in the formulations. This study concludes that an optimized density was detected for the formulations, as if their density is large then larger droplets will move faster to the bottom while if their density is small then larger droplets will move faster to the top.

Spreadability: The formulations presented the spreadability in the range of 15.39 ± 0.86 g.cm/sec for Formulation-1 and 17.55 ± 0.64 g.cm/sec for Formulation-2 which reflected that the gel formulation can be easily spread by a small amount of shear. A relative study of spreadability and viscosity revealed that with an enhancement of formulation viscosity, the spreadability reduces significantly.

Extrudability: The formulated gel preparations (F1-F2) displayed a notable extrudability with a large volume of extrudes (++ to +++). With an increase in the viscosity of the formulation, the extrudability decreases alongside and prevents easy extrusion from the collapsible tube.

Swelling index: The swelling index was observed as $115 \pm 4\%$ for Formulation-1 and $113 \pm 5\%$ for Formulation-2. The swelling index signified the matrix nature of the gel formulation which facilitates a controlled release of the drug.

Accelerated Stability: On subjecting both the gel formulations (F1-F2) at accelerated conditions ($40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH) for 30 days, no substantial disparity in the pH, viscosity, spreadability, swelling index, extrudability, and physical appearance were detected. A change in pH by 0.1-0.2 unit, viscosity by 600-800 cps, swelling index by 9%-11%, and spreadability by 0.79 g.cm/sec-0.98 g.cm/sec have been noticed

considerably. However, no changes in the physical appearance, translucency, and smoothness have been seen after the study. Overall, the formulation remained stable for the 1-month duration and is expected to remain in its original form for a longer duration in tropical and sub-tropical regions.

Freeze-thaw study: Similarly, the freeze-thaw study highlighted results corresponding to the accelerated stability study. Minor changes in the key parameters such as pH, viscosity, swelling index, spreadability have been noticed considerably. However, no changes in the physical appearance, translucency, and smoothness have been seen after the study. It can be concluded that both the topical gel formulations passed the Freeze-Thaw test. In general, the formulation is predicted to last longer in tropical and sub-tropical regions in its original form.

CONCLUSION

The study concluded that both the plant extracts (*D. biflorus* and *C. intybus*) with prominent anti-inflammatory activities were successfully incorporated into the Carbopol 934-based topical gel formulations (F1 and F2). The formulations showed good visual appearance, partial transparency, obligatory homogeneity, free from stickiness, easy removal from the skin surface, brilliant washability, pH value equivalent to the skin, free from topical irritation, suitable thermal stability, passed the centrifugation test, moderate conductivity, optimized viscosity, density equivalent to aqua, limited swelling, required spreadability, desired extrudability, low amount of non-volatile component, and proper stability under accelerated conditions cum Freeze-thaw studies. As a result, it was concluded that these two developed formulations may be very effective solution for the treatment of various inflammatory diseases (either on a topical area or even in transdermal region). However, additional thorough pre-clinical studies, clinical evaluations as per standard protocols, and long-term (minimum 12 months) stability research are seldom required.

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