

**DEVELOPMENT AND EVALUATION OF TOPICAL ANTI-INFLAMMATORY GEL FORMULATIONS CONTAINING *PTEROCARPUS MARSUPIUM* STANDARDIZED BARK EXTRACT AND *CRATAEVA NURVALA* STANDARDIZED BARK EXTRACT****Kamini Kashyap\***

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**ABSTRACT**

On an individual capacity, both *Pterocarpus marsupium* and *Crataeva nurvala* have shown notable anti-inflammatory behavior in animal models. *P. marsupium* and *C. nurvala*'s synergistic combined *in vitro* anti-inflammatory activity was recently published, opening up new research avenues. As a result of this, we agreed to develop a topical gel solution that could be used to manage a variety of skin conditions. The aim of this study was to create two topical gel formulations (F1 and F2) with Carbopol 934, propylparaben, methylparaben, triethanolamine, and propylene glycol 400 as active ingredients. Visual consistency, viscosity, transparency, density, pH, removal test, non-volatile matter, irritation test, solubility, swelling index, homogeneity, extrudability, washability, spreadability, thermal stability, centrifugation test, freeze-thaw cycle, and accelerated stability were all used to assess the items. Both plant extracts (*P. marsupium* and *C. nurvala*) with strong anti-inflammatory properties were effectively integrated into carbopol-based topical gel formulations, according to the findings. As a consequence, it was determined that the formulation may be a very useful topical or transdermal remedy for inflammatory diseases. More preclinical, clinical, and long-term stability research is required, however.

**KEYWORDS:** *Pterocarpus marsupium*, *Crataeva nurvala*, Anti-inflammatory, Evaluation, Formulations, Topical Gel.**INTRODUCTION**

Skin inflammation is a sign of the body's immune response. Symptoms include redness, sweating, itching, sensitivity, and swelling. Acute skin inflammation, such as that caused by a skin infection, or chronic skin inflammation, such as that caused by an inflammatory condition like psoriasis, is also possible.<sup>[1]</sup> While the treatment is decided by the source of the inflammation, the majority of cases of skin inflammation are treatable. Skin infections occur as bacteria or other foreign particles enter the skin as a result of a cut or wound. People with compromised immune systems are more susceptible to skin diseases.<sup>[2]</sup> Diabetes, a lack of oxygen, old age, and obesity are also contributing factors. While some bacteria just harm a small area of skin, others may reach further into the skin's layers and even beyond. Cellulitis, impetigo, and staphylococcal infections are bacterial skin infections caused by bacteria entering the skin.<sup>[3]</sup> Infectious infections, such as shingles and warts, are caused by viruses. Fungal diseases caused by fungi penetrating the skin include athlete's foot and yeast infections. Finally, lice and scabies viruses trigger bacterial skin infections.<sup>[4]</sup>

Leucoderma, elephantiasis, diarrhea, cough, scalp discoloration, and rectalgia have also been treated with *Pterocarpus marsupium* in the past. It is non-toxic and effective in the treatment of jaundice, fever, burns, asthma, stomachaches, and ulcers. Flavonoids and polyphenolic compounds are abundant in *P. marsupium*. The active phytoconstituents are thermostable. Leaves for boils, sores, skin infections, and abdominal pain; flowers for fever; Gum-Kino for diarrhea, dysentery, leucorrhoea, and other diseases; and bark as an astringent and for toothache. Traditionally, decoctions of bark and resin have been used to treat gland tumors, urethral discharges, and as an abortifacient. Astringent, anti-inflammatory, anti-diabetic, and anodyne properties are all contained in the heartwood.<sup>[5]</sup>

*Crataeva nurvala* urinary problems, thyroid, bladder stones, fever, vomiting, and gastric discomfort all benefit from the bark. It is often used as contraception and an oxytocic, and the juice of the bark is supplied to women after they have given birth. The root and bark of this plant are laxatives, lithontriptics, and stimulants of appetite and bile secretion. Externally, the leaves are rubefacient and anti-rheumatic; mentally, they are a

febrifuge and tonic. The tree has been used for anti-periodic, waste prevention, and respiratory conditions, fever and metabolic diseases, joint lubrication, skin moisture, wound healing, memory failure, pulse, liver, and immune system fatigue in the past. The bark is used as an appetite stimulant and to reduce bile and phlegm secretion in the Unani method of medicine.<sup>[6]</sup>

On an individual capacity, both *P. marsupium* and *C. nurvala* have shown notable anti-inflammatory behavior in animal models.<sup>[7-10]</sup> *P. marsupium* and *C. nurvala*'s combined synergistic *in vitro* anti-inflammatory activity was recently published, opening up new research avenues. As a result of this, we agreed to develop a topical gel solution that could be used to manage a variety of skin conditions.<sup>[11]</sup>

The aim of this study was to fabricate two topical gel formulations (F1 and F2) that included both herbal and synthetic components by combining Carbopol 934, methylparaben, propylparaben, propylene glycol 400, and triethanolamine. Visual consistency, viscosity, transparency, density, pH, removal test, non-volatile matter, irritation test, solubility, swelling index, homogeneity, extrudability, washability, spreadability, thermal stability, centrifugation test, freeze-thaw cycle, and accelerated stability were all used to assess the products.

## MATERIALS AND METHODS

### Chemicals

The reagents, consumables, and chemicals for this research were obtained from HiMedia<sup>®</sup> India Pvt. Ltd., Mumbai, through a local distributor. S.A. Herbal Bioactive, Mumbai, Maharashtra, produced standardized (Tannins NLT 5% / 18:1) *P. marsupium* bark extract, and Green Heavens Private Limited, Nagpur, Maharashtra,

provided standardized (20% - 30% saponins) *C. nurvala* bark extract.

### Instruments

The water for the procedure came from a Borosil<sup>®</sup> (India) double-distilled water apparatus. The chemicals were measured using an Accro Tech<sup>®</sup> electronic balance (Model: AT-266-1, India). A digital pH meter (Contech<sup>®</sup>) was used to determine the pH of the solutions. The viscosity was measured using a Brookfield<sup>®</sup> viscometer (DV-III programmable Rheometer). A CentriBio<sup>®</sup> 80-2B centrifuge is used for the centrifugation. A Labtronics<sup>®</sup> digital conductivity meter (Model: ABS-1, India) was used to test conductivity, and a Bio-Technics<sup>®</sup> stability chamber was used for accelerated stability tests.

### Preparation of Formulations

1 g Carbopol 934 was distributed in 50 mL distilled water, and the beaker was set aside for half an hour to enable the contents to swell. Stirring was also required to combine the Carbopol 934 to achieve a gel quality. The needed amounts of preservatives (methylparaben and propylparaben) were dissolved in 5 mL of distilled water using a water bath. Propylene glycol 400 was applied after the solvent was cooled. The requisite amounts of standardized *P. marsupium* seed extract and standardized *C. nurvala* root extract were then added to the above mixture, and the volume was increased to 100 mL by adding the remaining purified water. Finally, the completely combined products were thoroughly blended into the Carbopol 934 gel with constant stirring. Finally, triethanolamine was applied to the formulation drop by drop to change the necessary skin pH (6.8-7.2) and achieve the desired gel consistency. The ingredients used to make this herbal topical gel substance are mentioned in Table 1.

**Table 1: Topical herbal gel formulations detail.**

INGREDIENTS	FORMULATION-1 (F1)	FORMULATION-2 (F2)
<i>Pterocarpus marsupium</i> Standardized Seeds Extract	1 g	0.5 g
<i>Crataeva nurvala</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 carried out according to the methods provided by Borkar *et al.*, 2020<sup>[12]</sup>; Mahajan *et al.*, 2017<sup>[13]</sup>; Mahajan *et al.*, 2017a<sup>[14]</sup>, and Shivhare *et al.*, 2019.<sup>[15]</sup>

#### Visual clarity

On a black and white backdrop, all of the formulated formulations were visually checked for transparency, appearance, color, and quality.

#### Transparency

The clarity of 5 mL of prepared gel in a 10 mL test tube was determined visually.

#### Stickiness

The stickiness was measured by applying a small amount of gel to the formulation and checking for the presence or lack of stickiness after it was added.

**Solubility**

At room temperature, the solubility of the gel was determined using a variety of solvents like water, methanol, propylene glycol, glycerin, acetone, and petroleum ether.

**pH**

The pH of the dermal gel was determined using a modified digital pH meter. Until achieving a constant reading, the glass electrode was dipped in 1 g of the formulation dissolved in 25 mL of distilled water. The pH was calculated three times for each formulation and the average was published.

**Conductivity**

The conductivity was calculated in millivolts (mV) and the glass electrode was calibrated utilizing the equipment's solutions (pH of 4.00 and 7.00). To reach equilibrium while measuring, the formulation was left for around 15 mins. The solution's conductivity was evaluated three times, with the average values calculated.

**Spreadability**

The slip-drag function was used to measure the spreadability of the polyherbal dermal gel. Inserting 2 g of formulation on a field slide and sandwiching it between equal glide slides with a hook attached was the procedure. A heavy mass was applied to the slides to remove the compressed air and form a standardized film between them. The remaining gel was scraped away from the corners. After that, the top slide was made to sag with a 50 g force. The period it took the top slide to cover a distance of 6 cm was calculated using the following 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 assessed by first adding the gel to the skin and then manually inspecting the result while evaluating the ease and degree of washing with purified water.

**Removal**

Through adding the gel to the skin and then wiping the applied portion with tissue paper, the ease of removal of the applied gel formulations was investigated.

**Extrudability**

To assess the extrudability of the prepared solution, 100 g of gels is filled into capped collapsible aluminum tubes and sealed with a manual ointment sealing device. The tubes (which included different formulations) were tightly clamped between two slides. The slides were then weighted with a 500 g weight, the cap was opened, and the extruded ribbon length was calculated after 10 mins.

**Viscosity**

The viscosity of the formulation was determined using a Digital Brookfield Viscometer at  $25 \pm 1^\circ\text{C}$  and 10 rpm on spindle no. 6. Before the tests, a sufficient quantity of gel was filled in a fitting big mouth jar in such a way that the spindle can be dipped and allowed to settle for 30 mins.

**Homogeneity**

Many of the formulated formulations were visually inspected for homogeneity (appearance, presence of any aggregates, kind of stain, after-feel, and removal of gel) after the gel had stabilized in suitable beakers.

**Thermal stability**

With the aid of a spatula, the gel was inserted into a glass jar and tapped to settle at the bottom. The bottle was filled two-thirds full, with a plug-in place and the cap tightened. After being kept upright in the incubator at  $45^\circ\text{C}$  for 48 hours, the physical presence of this filled container was registered.

**Loss on Drying**

It is calculated using 1 g of the formulation dried for 3 hrs at  $105^\circ\text{C}$ . To examine molecules, mix them, and accurately weigh them. The samples were put in a jar, the lid was closed, and the bottle and contents were accurately determined by gentle, sideways shaking. The samples were spaced as evenly as possible to a depth of about 5 mm before being put in the drying chamber and dried at the specified temperature for constant weight. Until weighing, the chamber was automatically closed, allowing the container to reach room temperature in desiccators. The method for calculating drying loss is.

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

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 density of the formulations was determined using a pycnometer, which is a glass flask with a tight-fitting glass stopper with a capillary hole in it. Weighing a pycnometer before filling it with each gel formulation and weighing it again after filtering out any excess gel was used to balance it. The gel density was measured as follows.

$$\rho = m / v$$

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

**Freeze-Thaw Cycle**

A freeze-thaw period was applied to the gel sample, which took 12 days and six cycles to complete. Throughout each series, the substance was held at a constant temperature for 24 hrs. The refrigerator was  $5 \pm 2^\circ\text{C}$ , and the greenhouse was  $40 \pm 2^\circ\text{C}$ .

**Centrifugation test**

10 g of the formulation was added to a tapered test tube, and the sample gel was centrifuged at 3000 rpm for 30 minutes at room temperature.

**Swelling index**

To determine the swelling index, 2 g of the prepared dermal herbal gel was put in a beaker containing 10 mL of distilled water. After 1 hr, the swelled formulation was removed from the beaker and put on a Petri dish. The weight of the contents was re-weighed, and the swelling index was calculated using the following 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 applied in a 0.5 g quantity to hairless skin over a 6 cm<sup>2</sup> region, then wrapped in a semi-occlusive bandage for 1 hr. After the care time had ended, the bandage was removed, the applied gel was scraped off completely, and the area was visually inspected for any rashes or other symptoms. The research took place over the course of seven days. The results were expressed using grades.

**Non-Volatile Matter**

1-5 g of the formulated gel was weighted and heated on a steam bath until any of the explosive matter had leaked in an evaporating dish. After 2 hrs of heating at 105°C in an oven and cooling in desiccators, the weight was taken. The method of heating, cooling, and weighing was replicated until the mass difference between two consecutive weights was less than 1 mg. The following is the approximation formula.

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

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**

The formulation was subjected to a 30-day rapid stability study in a stability chamber (40°C ± 2°C temperature; 75% ± 5% relative humidity). The formulated gel solution was held in a black foil-covered polyvinyl chloride container. We have found out what the critical criteria are.

**Statistical analysis**

After experimenting three times, the findings were calculated as mean standard deviation and statistically evaluated using a t-test, with  $p < 0.05$  deemed meaningful.

**RESULTS AND DISCUSSION****Evaluation of Gel Formulations****Visual clarity**

The two gel formulations (F1-F2) had a pale brown, less translucent, hazily clear, smooth textured look with no firm particles or grittiness. Overall, the formulas have

desirable organoleptic properties and attractive appearances, and they could be suitable to patients (or high patient compliance).

**Transparency**

The formulations were not transparent at all, and the final products have a low level of transparency. The amount of carbopol 940 in the gel formulations has a big impact on this translucency property (F1-F2). The clarity of the gel formulations improves with a lower percentage of carbopol 940.

**Homogeneity**

There were no firm objects or grittiness when touched between the fingertips, indicating a strong degree of homogeneity of both the formulations (F1-F2). A high degree of homogeneity revealed the quality attributes' superiority and, finally, their mass acceptability.

**Stickiness**

The findings showed that the prepared gels (F1-F2) were free of stickiness and distributed easily on the skin after application. These characteristics result in simple application across the skin surface, no stickiness with clothing, easy removal, and improved patient conformity and acceptability.

**Removal**

The study found that tissue paper would safely strip all topical herbal gel formulations (F1-F2) without leaving any marks on the scalp. This function allows patients to exclude formulations accidentally if they get irritated or if they no longer want them.

**Washability**

All of the developed formulations have a fantastic washability characteristic (F1-F2). Under unintended circumstances, this function allows the patient to quickly wash off the formulations of his or her own free will from the application field. This improves the patient's willingness to use the herbal topical product that has been created.

**Non-volatile component**

For Formulation-1 and Formulation-2, the non-volatile portion (such as terpenes and other small compounds) was found to be 10.74 % w/w and 10.19 % w/w, respectively. The volatile components (such as steroids, ellagitannins, flavonoids, saponins, phenolic resins, and alkaloids) for Formulation-1 and Formulation-2 were determined to be 89.26 % w/w and 89.81 percent w/w, respectively, which escaped from the substance after heating at 105°C for 2 hrs.

**Centrifugation test**

The centrifugation test showed that none of the established formulations (F1-F2) were unstable. According to the findings, the gel formulas are very robust and may not exhibit any physical defects such as phase splitting or frothing.

### **Thermal test**

Both formulations passed a thermal inspection, indicating that they are stable under heat stress. In both gel formulations (F1-F2), no phase splitting, fracturing, cracking, migration, or frothing is observed. Even though the topical gel goods are stable at high temperatures for long periods of time, it is highly recommended that they be kept in a cold, dry spot.

### **Solubility**

Both gel formulations (F1-F2) were virtually insoluble in pure water, slightly soluble in methanol, and fully soluble in propylene glycol, glycerin, acetone, and petroleum ether during the solubility study. As a result of this, the formulation ingredients and herbal components (especially phytoconstituents) the gel formulation contains a lot of non-polar components whereas polar components are almost non-existent.

### **Loss on drying**

The drying loss was below the prescribed limits (not more than 0.5%). Formulation-1 has a drying loss of  $0.17\pm 0.04\%$ , while Formulation-2 has a loss of  $0.24\pm 0.06\%$ . This parameter determined that the formulations provide sufficient moisture / humidity to shape a gel film to provide a humid atmosphere to the inflamed field.

### **Irritation test**

No skin discomfort, edema, rashes, erythema, or other dermatological reaction or specific inflammation is observed after 7 days of application of the gel formulations (F1-F2). As a result, the product is considered exceptionally effective for topical use.

### **pH**

The pHs of Formulation-1 and Formulation-2 gel formulations are observed to be  $6.98\pm 0.3$  and  $7.12\pm 0.2$ , respectively, which is within the standard pH range of the skin. This parameter indicates that the formulations would not cause the patients any annoyance, pain, or discomfort.

### **Conductivity**

Formulation-1 had a conductivity of  $62.67\pm 3.87$  mV, while Formulation-2 had a conductivity of  $70.82\pm 3.11$  mV. The gel has a low to moderate conductivity, implying that it should only be used on the skin and is not designed for any special ultrasound transmitting applications, or in other words, internal applications.

### **Viscosity**

Viscosity is a critical factor that affects pharmaceutical properties such as spreadability, extrudability, and bottle pourability, among others. The viscosity of the formulations is between  $39400\pm 700$  cps for Formulation-1 and  $47600\pm 800$  cps for Formulation-2. The rheological study found that as torque is increased, the shear stress rises significantly, resulting in a decrease in formulation viscosity.

### **Density**

Both formulas were subjected to density measurements with a pycnometer. Due to the strong viscosity of the formulations, filling the pycnometer was extremely challenging. As a consequence of the strong aqueous content of the formulations, the densities of these formulations are close to those of water ( $1.157\pm 0.38$  g/cm<sup>3</sup> for Formulation-1 and  $1.143\pm 0.26$  g/cm<sup>3</sup> for Formulation-2). This research found that the formulations have an optimal density, since larger droplets would travel faster to the bottom if their density is high, whereas smaller droplets will move faster to the top if their density is low.

### **Spreadability**

Formulation-1 had a spreadability of  $14.08\pm 0.44$  g.cm/sec, while Formulation-2 had a spreadability of  $16.12\pm 0.72$  g.cm/sec, indicating that the gel formulation can be quickly spread through a slight volume of shear. A comparison of spreadability and viscosity showed that as the viscosity of the solution increases, the spreadability decreases dramatically.

### **Extrudability**

The extrudability of the formulated gel preparations (F1-F2) was noteworthy, with a significant amount of extrudes (++ to +++). The extrudability of the formulation reduces as the viscosity of the formulation rises, preventing easy extrusion from the collapsible tube.

### **Swelling index**

Formulation-1 had a swelling index of  $123\pm 3$  percent, while Formulation-2 had a swelling index of  $117\pm 4$  percent. The swelling index denoted the gel formulation's matrix design, which allows for regulated drug release.

### **Accelerated Stability**

After 30 days of exposure to accelerated conditions ( $40\pm 2^\circ\text{C}$  and  $75\pm 5\%$  RH), no significant differences in pH, viscosity, spreadability, swelling index, extrudability, and physical appearance were observed for the formulations (F1-F2). A 0.2-0.3 unit shift in pH, 800-1100 cps viscosity, 10-13% swelling index, and 0.86-1.03 g.cm/sec spreadability have also been noted. After the analysis, no improvements in physical appearance, translucency, or smoothness were observed. Overall, the formulation stayed constant for a month and is predicted to continue in its original shape in tropical and sub-tropical regions for longer.

### **Freeze-thaw study**

Similarly, the freeze-thaw analysis brought up findings that were similar to those seen in the accelerated stabilization study. There have been significant improvements in core parameters such as pH, viscosity, swelling index, and spreadability. After the analysis, no improvements in physical appearance, translucency, or smoothness were observed. The Freeze-Thaw procedure was successfully completed by both topical gel

formulations. In general, the formulation is expected to survive longer in its original form in tropical and sub-tropical areas.

### CONCLUSION

Both plant extracts (*P. marsupium* and *C. nurvala*) with strong anti-inflammatory properties were effectively integrated into Carbopol 934-based topical gel formulations, according to the findings (F1 and F2). The formulations had a good visual appearance, partial transparency, no topical irritation, suitable thermal stability, brilliant washability, optimized viscosity, pH value equivalent to skin, required homogeneity, limited swelling, passed the centrifugation test, density equivalent to aqua, moderate conductivity, no stickiness, easy removal from the skin surface, and required stability. As a consequence, it was determined that these two newly formulated compounds may be a very successful therapy for a variety of inflammatory diseases (either on a topical area or even in transdermal region). However, additional comprehensive pre-clinical trials, routine clinical assessments, and long-term (minimum 12 months) stability testing are rarely needed.

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### CONFLICT OF INTEREST

The authors declare no Conflict of Interest regarding the publication of the article.

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