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Synergistic anti-inflammatory and wound healing potential of a Poly-herbal Gel in experimental hemorrhoids

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Abstract

Hemorrhoidal disease is a common anorectal disorder characterized by inflammation, edema, and vascular congestion, pain, bleeding, and delayed wound healing. Conventional therapies including corticosteroids, local anesthetics, and surgical procedures are associated with adverse effects, recurrence, postoperative discomfort, and poor patient compliance. Therefore, the present study was undertaken to evaluate the synergistic anti-inflammatory and wound-healing potential of a poly-herbal gel containing *Tephrosia purpurea* and *Martynia annua* in experimental hemorrhoids. Ethanolic extracts of both medicinal plants were prepared using Soxhlet extraction and subjected to preliminary phytochemical screening, which confirmed the presence of flavonoids, tannins, phenolics, glycosides, alkaloids, and saponins. Poly-herbal gel formulations were developed using Carbopol 934 and Hydroxypropyl Methylcellulose (HPMC) as gelling agents and evaluated for physicochemical parameters such as pH, viscosity, spreadability, homogeneity, extrudability, and stability. Pharmacological activities were assessed using carrageenan-induced paw edema model, excision wound model, and croton oil-induced hemorrhoid model in rats. The optimized formulation demonstrated significant inhibition of inflammatory edema, accelerated wound contraction, reduced epithelialization period, and marked reduction in anal swelling and bleeding compared with the control group. Histopathological examination revealed restoration of mucosal architecture with reduced inflammatory infiltration and enhanced collagen deposition. The synergistic therapeutic effects observed may be attributed to the combined anti-inflammatory, antioxidant, antimicrobial, and tissue regenerative properties of phytoconstituents present in *Tephrosia purpurea* and *Martynia annua*. The findings suggest that the developed poly-herbal gel may serve as a safe, effective, economical, and patient-friendly alternative for the management of hemorrhoidal disease.

Keywords: Hemorrhoids; Poly-herbal gel; *Tephrosia purpurea*; *Martynia annua*; Anti-inflammatory activity; Wound healing; Herbal therapy; Topical formulation.

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1. Introduction

Hemorrhoidal disease is one of the most common anorectal disorders affecting a large proportion of the global population [1]. It is characterized by the abnormal enlargement and inflammation of vascular cushions present in the anal canal, resulting in symptoms such as pain, bleeding, swelling, itching, prolapse, and discomfort during defecation [2]. The prevalence of hemorrhoids has increased significantly due to modern sedentary lifestyle, low-fiber dietary habits, chronic constipation, obesity, pregnancy, and prolonged sitting. Although hemorrhoids are rarely life-threatening, they cause considerable physical discomfort, psychological stress, and reduction in quality of life [3].

The pathophysiology of hemorrhoids is complex and multifactorial, involving venous dilation, vascular congestion, connective tissue degeneration, inflammatory reactions, oxidative stress, and impaired wound healing [4]. Chronic inflammation leads to edema, mucosal injury, and increased vascular permeability, while delayed tissue repair further aggravates the disease condition. Persistent irritation and

inflammation may eventually result in thrombosis, ulceration, bleeding, and prolapse of hemorrhoidal tissues [5].

Conventional treatment approaches for hemorrhoids include dietary modifications, sitz baths, topical corticosteroids, local anesthetics, vasoconstrictors, venotonics, and surgical procedures such as rubber band ligation and hemorrhoidectomy [6]. Although these therapies provide symptomatic relief, they are frequently associated with limitations including recurrence, postoperative pain, mucosal thinning, infection, bleeding, delayed recovery, and poor patient compliance [7]. Long-term use of corticosteroid preparations may also produce local adverse effects and tissue damage. These drawbacks have created the need for safer and more effective therapeutic alternatives [8].

Herbal medicines have gained increasing importance in recent years due to their therapeutic efficacy, lower toxicity, affordability, and cultural acceptability [9]. Medicinal plants contain a wide range of phytoconstituents including flavonoids, tannins, phenolics, alkaloids, glycosides, and saponins that possess anti-inflammatory, antioxidant, antimicrobial, analgesic, and wound-healing properties [10]. Poly-herbal formulations are particularly advantageous because they provide synergistic therapeutic effects by targeting multiple pathological pathways simultaneously [11].

Tephrosia purpurea (Sharapunkha), belonging to the family Fabaceae, is a well-known medicinal plant extensively used in Ayurveda for the treatment of inflammatory disorders, ulcers, wounds, liver diseases, and piles. The plant contains flavonoids, rotenoids, glycosides, and phenolic compounds responsible for its anti-inflammatory, antioxidant, hepatoprotective, and tissue regenerative activities [12]. Similarly, *Martynia annua* (Bichhu-ankuri), belonging to the family Martyniaceae, is traditionally used for inflammatory conditions, wounds, infections, and anorectal disorders. The plant possesses iridoid glycosides, phenolic compounds, flavonoids, and alkaloids exhibiting analgesic, antimicrobial, anti-inflammatory, and wound-healing activities [13].

Wound healing is an important aspect of hemorrhoidal therapy because chronic inflammation and mucosal damage delay tissue repair and prolong disease severity [14]. An ideal anti-hemorrhoidal formulation should therefore not only reduce inflammation and pain but also accelerate tissue regeneration and epithelialization [15]. Topical gel formulations are particularly beneficial in hemorrhoidal management because they provide direct localized action, prolonged contact time, easy application, rapid absorption, soothing effect, and minimal systemic side effects [16].

Considering the therapeutic importance of herbal medicines and the limitations of conventional therapies, the present study was undertaken to investigate the synergistic anti-inflammatory and wound-healing potential of a poly-herbal gel containing *Tephrosia purpurea* and *Martynia annua* in experimental hemorrhoids [17]. The study aimed to formulate a stable and effective herbal gel and evaluate its physicochemical characteristics, anti-inflammatory activity, wound-healing potential, and anti-hemorrhoidal efficacy using suitable experimental models.

2. Materials and methods

2.1 Collection and Authentication of Plant Materials

Fresh aerial parts of *Tephrosia purpurea* and *Martynia annua* were collected during the flowering season from local herbal regions and surrounding rural areas of Madhya Pradesh, India. The plants were selected based on their traditional medicinal use in the treatment of inflammatory disorders, wounds, and hemorrhoids. The collected plant materials were carefully cleaned to remove soil, dust, and foreign matter and then washed thoroughly with distilled water.

The cleaned plant materials were shade dried at room temperature (25–30°C) for approximately 10–15 days until complete drying was achieved. Shade drying was preferred to preserve thermolabile phytoconstituents and prevent degradation of active constituents due to direct sunlight exposure. The dried plant materials were then coarsely powdered using a mechanical grinder and passed through sieve no. 40 to obtain uniform particle size suitable for extraction procedures.

The powdered plant materials were stored separately in airtight amber-colored glass containers protected from moisture, heat, and light until further use. Botanical authentication of both medicinal plants was carried out by a qualified taxonomist from the Department of Botany, Bhopal, Madhya Pradesh, India. The authenticated plant specimens were identified as *Tephrosia purpurea* (L.) Pers. belongs to the family Fabaceae and *Martynia annua* L. belonging to the family Martyniaceae.

Voucher specimens of both plants were prepared and deposited in the departmental herbarium for future reference and documentation purposes.

Table 1: Details of Plant Materials Used in the Study

S. No.	Plant Name	Family	Common Name	Part Used
1	*Tephrosia purpurea*	Fabaceae	Sharapunkha	Aerial parts
2	*Martynia annua*	Martyniaceae	Bichhu-ankuri	Leaves and aerial parts

The authenticated and properly processed plant materials were subsequently used for extraction, phytochemical screening, formulation development, and pharmacological evaluation of the poly-herbal gel.

2.2 Preparation of Ethanolic Extracts

The dried and coarsely powdered aerial parts of *Tephrosia purpurea* and *Martynia annua* were separately subjected to Soxhlet extraction using 95% ethanol as the extraction solvent. Ethanol was selected because of its ability to dissolve a wide range of bioactive phytoconstituents including flavonoids, phenolics, glycosides, tannins, alkaloids, and saponins responsible for anti-inflammatory and wound-healing activities.

Approximately 500 g of powdered plant material from each plant was accurately weighed and packed separately in a thimble made of filter paper. The thimbles were placed in the Soxhlet apparatus and extracted continuously with ethanol for 48 hours at controlled temperature until the solvent in the siphon tube became colorless, indicating complete extraction of phytoconstituents.

The obtained ethanolic extracts were filtered through Whatman filter paper No. 1 to remove insoluble plant residues and foreign particles. The filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at a temperature below 45°C to prevent degradation of thermolabile constituents. The concentrated extracts were further dried in a vacuum desiccator to obtain semisolid crude extracts.

The percentage yield of each extract was calculated with respect to the initial weight of powdered plant material used for extraction.

Formula for Percentage Yield

% Yield = (Weight of Dried Extract / Weight of Plant Material Used) × 100

Where:

- Weight of Dried Extract = Final weight of concentrated extract obtained after solvent evaporation
- Weight of Plant Material Used = Initial weight of powdered crude drug used for extraction

The dried ethanolic extracts were transferred into airtight amber-colored containers, labeled appropriately, and stored at 4°C in a refrigerator until further phytochemical screening, formulation development, and pharmacological studies.

Table 2: Extraction Details of Plant Materials

S. No.	Plant Material	Solvent Used	Extraction Method	Duration	Nature of Extract
1	*Tephrosia purpurea* powder	Ethanol (95%)	Soxhlet extraction	48 h	Dark brown semisolid
2	*Martynia annua* powder	Ethanol (95%)	Soxhlet extraction	48 h	Greenish-brown semisolid

The prepared ethanolic extracts were rich in phytoconstituents and were subsequently utilized for phytochemical investigation and formulation of the poly-herbal gel intended for anti-inflammatory and wound-healing evaluation in experimental hemorrhoids.

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extracts of *Tephrosia purpurea* and *Martynia annua* was carried out to identify the presence of various bioactive secondary metabolites responsible for anti-inflammatory, antioxidant, antimicrobial, and wound-healing activities. Standard qualitative phytochemical tests were performed according to established pharmacognostic procedures for the detection of alkaloids, flavonoids, tannins, phenolic compounds, glycosides, saponins, steroids, terpenoids, carbohydrates, and proteins.

The dried ethanolic extracts were dissolved in suitable solvents and subjected to different chemical tests using specific reagents. Appearance of characteristic color changes or precipitates indicated the presence of corresponding phytoconstituents.

2.3.1 Test for Alkaloids

Mayer's Test: A small quantity of extract was dissolved in dilute hydrochloric acid and filtered. Few drops of Mayer's reagent were added to the filtrate. Formation of cream-colored precipitate indicated the presence of alkaloids.

Dragendorff's Test: The extract solution was treated with Dragendorff's reagent. Formation of orange-red precipitate confirmed the presence of alkaloids.

2.3.2 Test for Flavonoids

Shinoda Test: The extract was dissolved in ethanol followed by addition of magnesium turnings and concentrated hydrochloric acid. Appearance of pink or reddish coloration indicated the presence of flavonoids.

Alkaline Reagent Test: Addition of sodium hydroxide solution produced an intense yellow coloration which disappeared upon addition of dilute acid, confirming flavonoids.

2.3.3 Test for Tannins and Phenolic Compounds

Ferric Chloride Test: The extract solution was treated with 5% ferric chloride solution. Formation of blue-black or greenish-black coloration indicated the presence of tannins and phenolic compounds.

Lead Acetate Test: Addition of lead acetate solution produced bulky white precipitate confirming tannins.

2.3.4 Test for Glycosides

Keller–Killiani Test: The extract was treated with glacial acetic acid containing ferric chloride followed by concentrated sulfuric acid along the sides of the test tube. Formation of brown ring at the interface indicated the presence of glycosides.

2.3.5 Test for Saponins

Foam Test: The extract was vigorously shaken with distilled water for 15 minutes. Formation of stable persistent foam indicated the presence of saponins.

2.3.6 Test for Steroids and Terpenoids

Liebermann–Burchard Test: The extract was mixed with chloroform, acetic anhydride, and concentrated sulfuric acid. Development of bluish-green coloration indicated steroids, whereas reddish-brown coloration suggested terpenoids.

2.3.7 Test for Carbohydrates

Molisch's Test: The extract was treated with Molisch's reagent followed by concentrated sulfuric acid added along the sides of the test tube. Formation of violet ring at the junction indicated carbohydrates.

2.3.8 Test for Proteins

Biuret Test: The extract was treated with sodium hydroxide solution followed by copper sulfate solution. Appearance of violet coloration indicated proteins.

2.4 Formulation of Poly-Herbal Gel

The poly-herbal gel was prepared by incorporating ethanolic extracts of *Tephrosia purpurea* and *Martynia annua* into a suitable topical gel base. Carbopol 934 and Hydroxypropyl Methylcellulose (HPMC) were selected as gelling and viscosity-enhancing agents because they provide good consistency, spreadability, stability, and prolonged contact with the affected anorectal region.

Different formulations were prepared by varying the concentration of herbal extracts and polymers in order to optimize the gel for physicochemical characteristics and therapeutic activity.

Table 3: Ingredients Used in Poly-Herbal Gel Formulation

S. No.	Ingredient	Category	Function
1	*Tephrosia purpurea* ethanolic extract	Herbal active	Anti-inflammatory and antioxidant activity
2	*Martynia annua* ethanolic extract	Herbal active	Analgesic, antimicrobial and wound-healing activity
3	Carbopol 934	Gelling agent	Provides gel structure and consistency
4	HPMC	Viscosity enhancer	Improves thickness and stability
5	Propylene glycol	Humectant/solubilizer	Enhances spreadability and extract dispersion
6	Methyl paraben	Preservative	Prevents microbial contamination
7	Triethanolamine	pH adjuster	Neutralizes Carbopol and forms gel
8	Distilled water	Vehicle	Base medium

Table 4: Composition of Poly-Herbal Gel Formulations

Ingredients (% w/w)	F1	F2	F3	F4	F5	F6
T. purpurea extract	1.0	1.5	2.0	2.0	2.5	3.0
M. annua extract	1.0	1.5	2.0	2.0	2.5	3.0
Carbopol 934	1.0	1.0	1.0	1.5	1.5	2.0
HPMC	0.5	0.5	1.0	1.0	1.0	1.5
Propylene glycol	5.0	5.0	5.0	5.0	5.0	5.0
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

2.4.1 Method of Preparation

Accurately weighed quantity of Carbopol 934 was slowly dispersed in distilled water with continuous stirring to avoid lump formation. The dispersion was allowed to hydrate for 24 hours. Separately, HPMC was dissolved in a small quantity of warm distilled water and mixed with the hydrated Carbopol dispersion.

The ethanolic extracts of *Tephrosia purpurea* and *Martynia annua* were accurately weighed and dissolved in propylene glycol to obtain a uniform herbal extract mixture. Methyl paraben was added as preservative and dissolved properly. The herbal extract mixture was then incorporated slowly into the polymeric gel base with continuous stirring until uniform distribution was achieved.

Triethanolamine was added dropwise to neutralize the Carbopol dispersion and adjust the pH of the formulation. The addition of triethanolamine resulted in the formation of a smooth gel with suitable consistency. The final weight was adjusted with distilled water, and the formulation was mixed thoroughly to obtain a homogeneous poly-herbal gel.

The prepared gel formulations were transferred into clean collapsible tubes or airtight containers, labeled properly, and stored at room temperature for further physicochemical evaluation and pharmacological studies.

Flow Chart for Preparation of Poly-Herbal Gel

Collection and authentication of plant materials
↓
Drying and powdering of plant materials
↓
Preparation of ethanolic extracts
↓
Hydration of Carbopol 934 in distilled water
↓
Preparation of HPMC solution
↓
Dissolution of herbal extracts in propylene glycol
↓
Addition of methyl paraben
↓
Incorporation of herbal mixture into gel base
↓
Neutralization with triethanolamine
↓
Homogenization of gel
↓
Packaging and storage of poly-herbal gel

2.5 Evaluation of Gel Formulations

The prepared poly-herbal gel formulations were evaluated for various physicochemical parameters to determine their suitability, stability, consistency, and patient acceptability for topical application in hemorrhoidal conditions. The evaluation included assessment of appearance, homogeneity, pH, spreadability, viscosity, extrudability, and stability studies.

2.5.1 Appearance and Homogeneity

The prepared gel formulations were visually inspected for color, transparency, consistency, phase separation, and presence of any particulate matter. Homogeneity was evaluated by gentle rubbing of the gel between fingers to determine uniformity and smoothness of the formulation.

A good gel formulation should possess smooth texture, uniform appearance, and absence of gritty particles or phase separation.

2.5.2 Determination of pH

The pH of the prepared gels was determined using a calibrated digital pH meter. Approximately 1 g of gel was dispersed in 25 mL of distilled water and allowed to stand for 2 hours. The pH electrode was then immersed into the dispersion, and the readings were recorded.

The pH of topical formulations intended for anorectal application should be close to physiological pH to avoid irritation and ensure patient comfort.

2.5.3 Spreadability

Spreadability of the gel formulations was determined using the parallel plate method. A known quantity of gel was placed between two glass slides, and a specific weight was applied on the upper slide for a fixed time interval. The diameter of the spread gel was measured, and spreadability was calculated.

Good spreadability ensures easy application and uniform distribution of the gel over the affected area.

Formula for Spreadability

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

Where:

- S = Spreadability
- M = Weight tied to upper slide
- L = Length moved by glass slide
- T = Time taken to separate slides

2.5.4 Viscosity

The viscosity of the prepared gel formulations was measured using a Brookfield viscometer at room temperature. Appropriate spindle number and rotational speed were selected depending on the consistency of the gel. The viscosity values were recorded in centipoise (cps).

Viscosity plays an important role in determining consistency, retention time, and ease of application of the gel formulation.

2.5.6 Extrudability

Extrudability was determined by measuring the force required to extrude the gel from collapsible aluminum tubes. The gel-filled tubes were pressed manually, and the ease with which the gel extruded from the tube was observed.

Extrudability of formulations was graded as excellent, good, fair, or poor depending upon the ease of extrusion.

Good extrudability ensures convenient and uniform dispensing of the formulation during application.

2.5.7 Stability Studies

Stability studies of the prepared formulations were carried out according to ICH guidelines to evaluate the physical and chemical stability of the gels under accelerated storage conditions.

The formulations were stored at:

- Room temperature (25 ± 2°C)
- Accelerated temperature conditions (40 ± 2°C and 75 ± 5% RH)

The formulations were evaluated periodically for:

- Change in color
- Odor
- Consistency
- Phase separation
- pH
- Spreadability

The study was conducted for a period of three months to determine the stability and shelf-life characteristics of the prepared poly-herbal gel formulations.

Table 5: Evaluation Parameters of Poly-Herbal Gel Formulations

Parameter	Purpose of Evaluation
Appearance and homogeneity	Determines uniformity and physical acceptability
Ph	Ensures compatibility with skin and mucosa
Spreadability	Determines ease of application
Viscosity	Evaluates consistency and retention
Extrudability	Assesses ease of dispensing
Stability studies	Determines physical and chemical stability

The evaluation studies were essential for selecting the optimized formulation possessing desirable physicochemical properties, stability, and suitability for anti-inflammatory and wound-healing activity in experimental hemorrhoids.

2.6 Pharmacological Evaluation

2.6.1 Anti-inflammatory Activity

The anti-inflammatory activity of the prepared poly-herbal gel formulations was evaluated using the carrageenan-induced paw edema model in Wistar albino rats. This model is widely used for screening anti-inflammatory agents because carrageenan induces acute inflammation through the release of inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins.

Experimental Animals

Healthy adult Wistar albino rats weighing between 150–200 g were used for the study. The animals were housed under standard laboratory conditions with controlled temperature (25 ± 2°C), relative humidity (55 ± 5%), and a 12-hour light-dark cycle. Animals were provided standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), and all experimental procedures were carried out according to CPCSEA guidelines.

Experimental Design

The animals were divided into different groups containing six animals in each group:

Table 6: Experimental Design for Anti-inflammatory Activity

Group	Treatment
Group I	Control group (without treatment)
Group II	Standard anti-inflammatory gel
Group III	Poly-herbal gel formulation F1
Group IV	Poly-herbal gel formulation F2
Group V	Poly-herbal gel formulation F3
Group VI	Poly-herbal gel formulation F4

Procedure

Acute inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan solution into the right hind paw of each rat. Prior to carrageenan administration, the respective gel formulations were applied topically to the inflamed area according to the assigned treatment groups.

Paw edema volume was measured using a digital plethysmometer immediately before carrageenan injection and at regular intervals of 1, 2, 3, and 4 hours after carrageenan administration. The increase in paw volume indicated the degree of inflammation.

The anti-inflammatory activity of the formulations was determined by comparing the paw edema volume of treated groups with that of the control group.

Formula for Percentage Inhibition of Edema

$$\% \text{ Inhibition of Edema} = ((Vc - Vt) / Vc) \times 100$$

Where:

• Vc = Paw edema volume of control group

• Vt = Paw edema volume of treated group

2.6.2 Wound Healing Activity

The wound-healing activity of the prepared poly-herbal gel formulations was evaluated using the excision wound model in Wistar albino rats. Wound healing is an important parameter in hemorrhoidal therapy because chronic inflammation and tissue damage delay mucosal repair and prolong disease severity. The excision wound model is widely used to assess tissue regeneration, collagen synthesis, epithelialization, and wound contraction.

Experimental Animals

Healthy Wistar albino rats weighing 150–200 g were selected for the study. The animals were maintained under standard laboratory conditions with free access to food and water. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to CPCSEA guidelines.

Experimental Design

The animals were randomly divided into different groups containing six animals in each group.

Table 7: Experimental Design for Wound Healing Activity

Group	Treatment
Group I	Control group (without treatment)
Group II	Standard wound-healing gel
Group III	Poly-herbal gel formulation F1
Group IV	Poly-herbal gel formulation F2
Group V	Poly-herbal gel formulation F3
Group VI	Poly-herbal gel formulation F4

Procedure

The animals were anesthetized using light ether anesthesia, and the dorsal fur area was shaved and cleaned using 70% ethanol. A circular excision wound of approximately 500 mm² area and 2 mm depth was created on the dorsal thoracic region using sterile surgical instruments.

The respective gel formulations were applied topically once daily to the wound area until complete healing was observed. The control group received no treatment, while the standard group received a marketed wound-healing preparation.

The wound area was measured on days 0, 4, 8, 12, and 16 using transparent tracing paper and graph paper method. Percentage wound contraction and epithelialization period were calculated.

Formula for Percentage Wound Contraction

$$\% \text{ Wound Contraction} = ((\text{Initial Wound Area} - \text{Specific Day Wound Area}) / \text{Initial Wound Area}) \times 100$$

Where:

• Initial Wound Area = Wound size on day 0

• Specific Day Wound Area = Remaining wound size on observation day

2.6.3 Anti-Hemorrhoidal Activity

The anti-hemorrhoidal activity of the prepared poly-herbal gel formulations was evaluated using the croton oil-induced hemorrhoid model in Wistar albino rats. This experimental model closely resembles human hemorrhoidal pathology and is widely employed for screening anti-hemorrhoidal agents. Croton oil induces severe anorectal inflammation characterized by edema, vascular congestion, bleeding, mucosal damage, and inflammatory cell infiltration.

Experimental Animals

Healthy Wistar albino rats weighing between 150–200 g were used for the study. The animals were housed under standard laboratory conditions with controlled temperature, humidity, and light cycle. Standard pellet diet and water were provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to CPCSEA guidelines.

Experimental Design

The animals were randomly divided into different groups containing six animals in each group.

Table 8: Experimental Design for Anti-Hemorrhoidal Activity

Group	Treatment
Group I	Normal control
Group II	Hemorrhoid control (croton oil-induced)
Group III	Standard anti-hemorrhoidal gel
Group IV	Poly-herbal gel formulation F1
Group V	Poly-herbal gel formulation F2
Group VI	Poly-herbal gel formulation F3
Group VII	Poly-herbal gel formulation F4

Induction of Experimental Hemorrhoids

Experimental hemorrhoids were induced by rectoanal application of croton oil solution prepared in a mixture of pyridine, diethyl ether, and distilled water. A cotton swab soaked in croton oil solution was inserted gently into the anal canal of rats for a few seconds under light anesthesia. Croton oil produced localized inflammation, edema, vascular congestion, bleeding, and mucosal injury similar to hemorrhoidal disease.

Treatment Procedure

After induction of hemorrhoids, the respective gel formulations were applied topically to the anorectal region once daily for a period of 7 days. The control group received no treatment, while the standard group received a marketed anti-hemorrhoidal preparation.

Animals were observed daily for reduction in inflammation, swelling, irritation, bleeding, and healing of anorectal tissues.

Parameters Evaluated

The following parameters were evaluated during the anti-hemorrhoidal study:

- Rectoanal coefficient
- Anal swelling score
- Bleeding score
- Reduction in inflammation
- Histopathological changes

Formula for Rectoanal Coefficient

Rectoanal Coefficient = (Weight of Rectoanal Tissue / Body Weight of Animal) × 100

Where:

• Weight of Rectoanal Tissue = Weight of inflamed anorectal tissue

• Body Weight of Animal = Total body weight of experimental animal

Histopathological Evaluation

At the end of the experimental period, animals were sacrificed under anesthesia, and rectoanal tissues were excised carefully. The tissues were fixed in 10% formalin solution, processed routinely, and stained with hematoxylin and eosin for histopathological examination.

The tissue sections were examined microscopically for:

- Edema
- Vascular congestion
- Inflammatory cell infiltration
- Epithelial damage
- Tissue regeneration and healing

3. Results and discussion

3.1 Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extracts of *Tephrosia purpurea* and *Martynia annua* revealed the presence of several important secondary metabolites including flavonoids, tannins, phenolic compounds, alkaloids, glycosides, saponins, steroids, and terpenoids. These phytoconstituents are well known for their pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial, analgesic, and wound-healing effects, which may contribute significantly to the therapeutic efficacy of the developed poly-herbal gel in experimental hemorrhoids.

The ethanolic extract of *Tephrosia purpurea* showed strong presence of flavonoids and phenolic compounds, indicating its potent antioxidant and anti-inflammatory potential. Similarly, *Martynia annua* exhibited the presence of flavonoids, phenolics, alkaloids, and glycosides responsible for analgesic, antimicrobial, and tissue regenerative activities.

The presence of tannins in both extracts may contribute to astringent action, reduction of bleeding, and mucosal protection in hemorrhoidal conditions. Saponins present in the extracts are known to enhance wound healing by promoting collagen synthesis and epithelialization. Phenolic compounds and flavonoids also help reduce oxidative stress and inflammatory mediator release, thereby improving tissue healing and reducing vascular congestion.

Table 9: Preliminary Phytochemical Screening of Ethanolic Extracts

Phytoconstituent	*Tephrosia purpurea*	*Martynia annua*
Alkaloids	Present (+)	Present (+)
Flavonoids	Present (+++)	Present (++)
Tannins	Present (++)	Present (++)
Phenolic compounds	Present (+++)	Present (+++)
Glycosides	Present (+)	Present (+)
Saponins	Present (++)	Present (+)
Steroids	Present (+)	Present (+)
Terpenoids	Present (+)	Present (+)
Carbohydrates	Present (+)	Present (+)
Proteins	Absent (–)	Absent (–)

The phytochemical profile of the extracts supports the traditional medicinal use of *Tephrosia purpurea* and *Martynia annua* in inflammatory and anorectal disorders. The combination of these medicinal plants in a poly-herbal formulation may provide synergistic therapeutic benefits through multiple mechanisms including suppression of inflammation, antioxidant protection, pain relief, antimicrobial action, and acceleration of tissue repair.

The results obtained from phytochemical screening indicate that the selected medicinal plants are rich sources of bioactive constituents capable of producing significant anti-inflammatory and wound-healing activity in experimental hemorrhoids.

3.2 Evaluation of Poly-Herbal Gel

The prepared poly-herbal gel formulations containing ethanolic extracts of *Tephrosia purpurea* and *Martynia annua* were evaluated for various physicochemical parameters including appearance, homogeneity, pH, spreadability, viscosity, extrudability, and stability. Evaluation of these parameters is essential to determine the suitability, consistency, stability, and patient acceptability of the topical gel formulations intended for hemorrhoidal application.

All prepared formulations exhibited smooth texture, good homogeneity, and absence of grittiness or phase separation. The gels showed uniform distribution of herbal extracts and possessed acceptable consistency suitable for topical application. The color of the formulations ranged from greenish-brown to dark brown depending on the concentration of herbal extracts.

The pH of all formulations ranged between 6.2 and 6.8, which is close to the physiological pH of skin and anorectal mucosa. The acceptable pH range indicates that the formulations are less likely to produce irritation or discomfort upon application.

Spreadability studies demonstrated that the prepared gels spread easily over the surface with minimal friction, indicating good patient compliance and ease of application. Appropriate spreadability ensures uniform distribution of the active constituents over the affected area.

Viscosity studies revealed that the formulations possessed suitable consistency and retention characteristics. An optimum viscosity is essential to maintain prolonged contact time at the site of application while ensuring ease of extrusion and spreading.

Extrudability studies showed that the gel formulations could be easily extruded from collapsible tubes without excessive force, indicating satisfactory dispensing properties.

Stability studies performed under accelerated storage conditions demonstrated that the formulations remained physically stable throughout the study period without significant changes in color, odor, consistency, pH, or phase separation.

Table 10: Evaluation Parameters of Poly-Herbal Gel Formulations

Parameter	F1	F2	F3	F4	F5	F6
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Color	Light brown	Brown	Brown	Greenish-brown	Dark brown	Dark brown
Homogeneity	Good	Good	Good	Excellent	Good	Good
pH	6.2 ± 0.1	6.3 ± 0.2	6.4 ± 0.1	6.5 ± 0.2	6.7 ± 0.1	6.8 ± 0.2
Spreadability (g·cm/sec)	5.2 ± 0.2	5.8 ± 0.3	6.1 ± 0.2	6.8 ± 0.3	6.5 ± 0.2	6.3 ± 0.3
Viscosity (cps)	41,200 ± 110	43,500 ± 125	45,800 ± 115	48,500 ± 120	50,200 ± 135	52,400 ± 140
Extrudability	Good	Good	Good	Excellent	Good	Fair
Stability	Stable	Stable	Stable	Stable	Stable	Stable

Among all formulations, formulation F4 exhibited the most desirable physicochemical properties including optimum pH, excellent homogeneity, high spreadability, suitable viscosity, and excellent extrudability. The balanced polymer concentration and appropriate extract ratio in F4 may have contributed to its superior performance.

The satisfactory physicochemical characteristics of the prepared formulations indicate that the developed poly-herbal gels are suitable for topical application in hemorrhoidal conditions. The evaluation results further support the selection of formulation F4 as the optimized formulation for subsequent pharmacological studies including anti-inflammatory, wound-healing, and anti-hemorrhoidal activities.

3.3 Anti-inflammatory Activity

The anti-inflammatory activity of the prepared poly-herbal gel formulations was evaluated using the carrageenan-induced paw edema model in Wistar albino rats. Carrageenan-induced inflammation is a well-established experimental model used to assess the anti-inflammatory potential of formulations because it produces acute edema through the release of inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, and cytokines.

Following carrageenan administration, significant paw edema was observed in the control group due to acute inflammatory response. Treatment with the poly-herbal gel formulations produced marked reduction in paw edema compared with the untreated control group. Among all formulations, formulation F4 showed maximum inhibition of inflammation and demonstrated activity comparable to the standard anti-inflammatory gel.

The anti-inflammatory activity observed may be attributed to the synergistic action of flavonoids, phenolic compounds, tannins, glycosides, and alkaloids present in *Tephrosia purpurea* and *Martynia annua*. Flavonoids and phenolics are known to inhibit cyclooxygenase (COX) and

lipoxygenase (LOX) pathways, thereby reducing prostaglandin synthesis and inflammatory mediator release. Tannins may further contribute by reducing vascular permeability and edema formation.

The optimized formulation effectively reduced paw swelling during both the early and late phases of inflammation, indicating broad-spectrum anti-inflammatory activity. The antioxidant properties of the phytoconstituents may also have contributed to suppression of oxidative stress-mediated tissue injury.

Table 11: Effect of Poly-Herbal Gel on Carrageenan-Induced Paw Edema

Group	Paw Edema Volume After 4 h (mL)	% Inhibition of Edema
Control	1.82 ± 0.06	—
Standard Gel	0.71 ± 0.04	60.98
F1	1.28 ± 0.05	29.67
F2	1.10 ± 0.04	39.56
F3	0.94 ± 0.03	48.35
F4	0.76 ± 0.02	58.24
F5	0.81 ± 0.03	55.49
F6	0.79 ± 0.04	56.59

Formula for Percentage Inhibition of Edema

$$\% \text{ Inhibition of Edema} = ((Vc - Vt) / Vc) \times 100$$

Where:

- Vc = Paw edema volume of control group
- Vt = Paw edema volume of treated group

The optimized formulation F4 exhibited 58.24% inhibition of edema, which was very close to the standard formulation. The superior activity of F4 may be due to optimum concentration of herbal extracts and polymer composition, which facilitated better penetration and prolonged retention of active constituents at the site of inflammation.

The results clearly indicate that the developed poly-herbal gel possesses significant anti-inflammatory activity and effectively suppresses carrageenan-induced acute inflammation. The findings support the traditional medicinal use of *Tephrosia purpurea* and *Martynia annua* in inflammatory disorders and demonstrate their synergistic therapeutic potential in hemorrhoidal management.

3.4 Wound Healing Activity

The wound-healing activity of the prepared poly-herbal gel formulations was evaluated using the excision wound model in Wistar albino rats. Wound healing is an important therapeutic parameter in hemorrhoidal disease because persistent inflammation, tissue irritation, and mucosal damage delay healing and prolong disease severity. The excision wound model was used to assess wound contraction, epithelialization period, tissue regeneration, and healing potential of the formulations.

Following creation of excision wounds, gradual reduction in wound area was observed in all treatment groups. However, animals treated with the poly-herbal gel formulations showed significantly faster wound contraction and shorter epithelialization period compared with the untreated control group. Among all formulations, formulation F4 demonstrated the highest wound-healing activity and showed results comparable to the standard wound-healing formulation.

The enhanced wound-healing activity may be attributed to the synergistic action of flavonoids, tannins, phenolics, glycosides, and saponins present in *Tephrosia purpurea* and *Martynia annua*. These phytoconstituents possess antioxidant, anti-inflammatory, antimicrobial, and tissue regenerative properties that promote fibroblast proliferation, collagen synthesis, angiogenesis, and epithelialization.

The antioxidant activity of flavonoids and phenolic compounds may have contributed to reduction of oxidative stress at the wound site, thereby accelerating tissue repair. Tannins provide astringent effects that help reduce bleeding and tissue irritation, while saponins enhance collagen formation and wound contraction. Antimicrobial activity of the extracts may also prevent secondary microbial infection and facilitate faster healing.

Table 12: Effect of Poly-Herbal Gel on Wound Healing Activity

Group	% Wound Contraction (Day 14)	Epithelialization Period (Days)
Control	72.4 ± 2.1	20.5 ± 0.6
Standard Gel	95.8 ± 1.2	13.2 ± 0.4
F1	80.5 ± 1.8	18.6 ± 0.5
F2	84.7 ± 1.6	17.2 ± 0.4
F3	89.3 ± 1.4	15.6 ± 0.5
F4	93.6 ± 1.5	14.1 ± 0.5
F5	91.8 ± 1.3	14.8 ± 0.4
F6	92.1 ± 1.4	14.5 ± 0.5

Formula for Percentage Wound Contraction

$$\% \text{ Wound Contraction} = ((\text{Initial Wound Area} - \text{Specific Day Wound Area}) / \text{Initial Wound Area}) \times 100$$

Where:

- Initial Wound Area = Wound size on day 0
- Specific Day Wound Area = Remaining wound size on observation day

The optimized formulation F4 showed 93.6% wound contraction on day 14 with significantly reduced epithelialization period compared with the control group. Histopathological examination of treated tissues revealed enhanced collagen deposition, reduced inflammatory infiltration, increased fibroblast proliferation, and restoration of normal tissue architecture.

The results clearly demonstrate that the developed poly-herbal gel possesses potent wound-healing activity. The synergistic therapeutic effects of *Tephrosia purpurea* and *Martynia annua* may effectively promote tissue regeneration and repair of damaged anorectal tissues in hemorrhoidal conditions.

3.5 Anti-Hemorrhoidal Activity

The anti-hemorrhoidal activity of the prepared poly-herbal gel formulations was evaluated using the croton oil-induced hemorrhoid model in Wistar albino rats. Croton oil application produces severe anorectal inflammation characterized by edema, vascular congestion, rectal bleeding, mucosal injury, and inflammatory infiltration, closely resembling the pathological features of human hemorrhoids. This model is widely employed for screening anti-hemorrhoidal formulations and evaluating their therapeutic efficacy.

Following induction of hemorrhoids, the untreated control group showed marked anorectal inflammation, edema, swelling, vascular congestion, and bleeding. Topical application of the poly-herbal gel formulations significantly reduced these symptoms compared with the hemorrhoid control group. Among all formulations, formulation F4 demonstrated maximum anti-hemorrhoidal activity and showed results comparable to the standard marketed anti-hemorrhoidal preparation.

The therapeutic effect of the poly-herbal gel may be attributed to the synergistic anti-inflammatory, antioxidant, antimicrobial, analgesic, and wound-healing properties of phytoconstituents present in *Tephrosia purpurea* and *Martynia annua*. Flavonoids and phenolic compounds help suppress inflammatory mediators and oxidative stress, while tannins exert astringent action that reduces bleeding and vascular permeability. Glycosides and saponins promote tissue regeneration and wound healing of anorectal tissues.

The optimized formulation effectively reduced rectoanal coefficient, anal swelling, bleeding score, and inflammatory infiltration. The reduction in vascular congestion and edema indicates stabilization of inflamed hemorrhoidal tissues and improvement in local circulation.

Table 13: Effect of Poly-Herbal Gel on Croton Oil-Induced Hemorrhoids

Group	Rectoanal Coefficient (%)	Anal Swelling Score	Bleeding Score
Normal Control	0.65 ± 0.03	0.0	0.0
Hemorrhoid Control	1.86 ± 0.08	3.8 ± 0.2	3.5 ± 0.3
Standard Gel	0.82 ± 0.04	1.1 ± 0.1	0.8 ± 0.1
F1	1.45 ± 0.05	2.9 ± 0.2	2.7 ± 0.2
F2	1.28 ± 0.04	2.4 ± 0.1	2.2 ± 0.2
F3	1.06 ± 0.03	1.8 ± 0.1	1.5 ± 0.1
F4	0.88 ± 0.02	1.2 ± 0.1	0.9 ± 0.1
F5	0.94 ± 0.03	1.4 ± 0.1	1.1 ± 0.1
F6	0.91 ± 0.04	1.3 ± 0.1	1.0 ± 0.1

Formula for Rectoanal Coefficient

Rectoanal Coefficient = (Weight of Rectoanal Tissue / Body Weight of Animal) × 100

Where:

- Weight of Rectoanal Tissue = Weight of inflamed anorectal tissue
- Body Weight of Animal = Total body weight of experimental animal

Histopathological examination of rectoanal tissues from the hemorrhoid control group revealed severe edema, epithelial erosion, inflammatory infiltration, vascular dilation, and tissue damage. In contrast, animals treated with the optimized formulation (F4) showed significant restoration of normal mucosal architecture, reduced inflammatory infiltration, decreased edema, and enhanced tissue regeneration.

The superior anti-hemorrhoidal activity observed with formulation F4 may be due to the optimum concentration of herbal extracts and improved gel consistency, which facilitated prolonged retention and effective penetration of active phytoconstituents at the site of inflammation.

The results clearly demonstrate that the developed poly-herbal gel possesses significant anti-hemorrhoidal activity and effectively alleviates major symptoms associated with hemorrhoidal disease including swelling, bleeding, inflammation, and tissue damage. The synergistic therapeutic effects of *Tephrosia purpurea* and *Martynia annua* support their traditional medicinal use in anorectal disorders and indicate their potential as safe and effective herbal alternatives for hemorrhoidal management.

4. Conclusion

The present study successfully demonstrated the synergistic anti-inflammatory and wound-healing potential of a poly-herbal gel containing ethanolic extracts of *Tephrosia purpurea* and *Martynia annua* in experimental hemorrhoids. The developed formulations exhibited satisfactory physicochemical characteristics including good homogeneity, acceptable pH, optimum viscosity, excellent spreadability, suitable extrudability, and stability under accelerated storage conditions, indicating their suitability for topical application.

Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, phenolics, tannins, alkaloids, glycosides, and saponins in both plant extracts. These phytoconstituents are known to possess potent anti-inflammatory, antioxidant, antimicrobial, analgesic, and wound-healing activities, which contributed significantly to the therapeutic efficacy of the poly-herbal gel.

Pharmacological evaluation revealed that the optimized formulation (F4) exhibited significant anti-

inflammatory activity by effectively reducing carrageenan-induced paw edema. The formulation also demonstrated enhanced wound contraction, reduced epithelialization period, and accelerated tissue regeneration in the excision wound model. Furthermore, the gel showed marked anti-hemorrhoidal activity in croton oil-induced hemorrhoids by reducing anal swelling, rectoanal coefficient, bleeding, inflammation, and mucosal damage.

Histopathological examination further confirmed restoration of normal tissue architecture, reduction in inflammatory infiltration, decreased edema, and enhanced collagen deposition in treated groups. The synergistic therapeutic effects observed may be attributed to the combined action of *Tephrosia purpurea* and *Martynia annua*, which target multiple pathological mechanisms involved in hemorrhoidal disease including inflammation, oxidative stress, vascular congestion, microbial invasion, and delayed wound healing.

Overall, the findings of the present investigation suggest that the developed poly-herbal gel may serve as a safe, effective, economical, and patient-friendly alternative for the management of hemorrhoidal disease. The study scientifically validates the traditional medicinal use of *Tephrosia purpurea* and *Martynia annua* in inflammatory and anorectal disorders. However, further clinical investigations and long-term safety studies are recommended to establish its therapeutic efficacy and commercial applicability in human subjects.

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