Journal of Chemical Health Risks

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JCHR (2025) 15(6), 255-262 | ISSN:2251-6727

Development of anti-Inflammatory Hydrogel Incorporating Tulsi and Indian Frankincens for Enhanced Wound Healing and Tissue Repairing

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(Received: 27 September 2025 Revised: 05 October 2025 Accepted: 01 November 2025)

KEYWORDS

Ocimum sanctum, Boswellia serrata, Hydrogel, Anti-inflammatory, Wound healing, Tissue repair.

ABSTRACT:

The intricate biological process of wound healing is frequently hampered by microbial invasion and excessive inflammation. The current study focusses on creating and testing a polyherbal hydrogel that contains extracts of Boswellia serrata (Indian Frankincense) and Ocimum sanctum (tulsi), both of which have well-established anti-inflammatory and medicinal qualities. Phytochemical analysis was performed on the essential oil from Boswellia serrata gum resin and the ethanolic extract of Ocimum sanctum leaves that were obtained by steam distillation and Soxhlet extraction, respectively. Carbopol 940 was used as a gelling agent in the formulation of hydrogels, which were then optimised for physical properties such as appearance, pH, viscosity, homogeneity, spreadability, and drug content. The active ingredients showed a prolonged release profile in Franz diffusion cell-based in vitro release tests. The safety and therapeutic potential of the optimised formulation were validated by biological evaluations, such as protein denaturation-based anti-inflammatory assays and HET-CAM skin irritation. With no irritation or negative side effects, the produced hydrogel demonstrated strong anti-inflammatory effectiveness on par with regular diclofenac sodium. The robustness of the formulation under accelerated settings was further demonstrated by stability studies.

According to the results, a Tulsi–Frankincense hydrogel is a natural and safe substitute that can aid in tissue repair and wound healing. It may also be used as a topical medication.

Introduction

1. wound healing

Any disruption of living tissue integrity may be viewed as a wound. Skin is the largest organ of the human body and one of its primary duties is to shield water-rich interior organs from the dry exterior environment. [1] Maintaining skin integrity and developing a powerful wound healing capacity are critical criteria for healthy survival. Furthermore, wound healing can also offer a considerable difficulty and strain for health care systems. Medicare cost projections for acute and chronic wound treatments ranged from $28.1 billion to $96.8 billion during 2014. [2] The biggest wound-related expenses were due to surgical wounds followed by diabetic foot ulcers

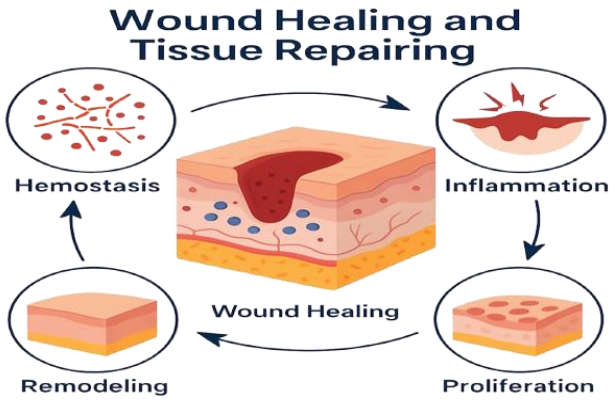

Figure 1 the process of wound healing and tissue repairing

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1.2 Inflammation

Inflammation is a protective response that triggers several physiological changes to minimise tissue damage and eliminate harmful pathogens. This method entails an intricate sequence of events, encompassing the widening of arterioles, venules, & capillaries, along with heightened vascular permeability. It also involves the release of fluids, including plasma proteins, and the movement of leukocytes into the inflamed region. [3,4].

The primary goal of inflammation is to eradicate and eliminate the harmful agent. However, if the procedure does not occur or is prolonged, inflammation will confine and localise the injury.

1.2 Acute inflammation

Refers to a brief period of inflammation that typically lasts from a few minutes to a few days

The primary attributes are:

- Fluids being released
- Plasma protein causes edoema, whereas leukocytes, particularly neutrophils, emigrate.

1.3 Chronic inflammation: Chronic inflammation is characterised by prolonged duration compared to acute inflammation. Histologically, this condition is distinguished by the presence of lymphocytes, macrophages, heightened angiogenesis, fibrosis, and necrosis. Active inflammation and tissue damage occur as a consequence of these processes. Subsequently, it is accompanied by severe inflammation and arises from a modest, slow-burning asymptomatic response. The acute inflammatory response is the rapid and immediate reaction that occurs in response to an infection or injury. It is a general response that the body uses as its first line of defence after being threatened [5]. Acute inflammation is distinguished by increased copper levels and decreased zinc levels. Leukocytosis, thrombocytosis, negative nitrogen balance, higher basal metabolic rate (BMR), increased lipogenesis, and improved lipolysis are observed. The plasma protein level has seen a decline, whereas the C reactive protein level has shown a rise. [6]

Advantages

- Hydrogel has superior elasticity and strength compared to other hydrogels with similar softness. The hydrogel implant material, composed of a copolymer of methyl acrylate and hydroxyethylacrylate, possesses both strength and softness.
- Hydrogel-based micro valves offer several advantages compared to conventional micro-valves, such as their relative simplicity.
- The device has the following characteristics: it is made through a manufacturing process, does not need an external power source, does not have any built-in electronic components, has a significant displacement of 185µm, and can generate a substantial force of 22 mn.
- Researchers are now studying natural hydrogel polymers for the purpose of tissue engineering. The components consist of agarose, methylcellulose, and other polymers that are produced from natural sources [7,8].

1.4 Applications of hydrogel

1. Wound healing: Hydrogels have the ability to retain water & medications due to their linked structure. Due to their moisture retention capabilities, they may effectively retain and contain fluids discharged from wounds. The gel is composed of either polyvinyl pyrrolidine or polyacrylamide, with a water concentration that varies between 70% and 95%. [9]

2 Material and Methods

2.1 Material

Plant materials:

Ocimum sanctum leaves & Indian frankincense gum resin were Procured from online (Order no: OD334651588854764100 & OD334953614945411100) respectively during the experimental work.

2.1.1 Collection of plant

Ocimum sanctum leaves were collected form market during experimental work, the Indian frankincense procured form the local market, cleaned, used for oil extraction**.**

2.2.1 Extraction Process

2.2.1.1 Extraction of *Ocimum sanctum*

During the experiment, leaves of Ocimum sanctum were procured from the market, and Indian frankincense was acquired from a local vendor, thereafter cleaned

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and employed for oil extraction. Sigma Aldrich is the source of PG-400, methylparaben, propylene glycol, polysorbate 80, myeloperoxidase (MPO), and propylparaben Carbopol. **[10-12]**

Figure: 2 **Soxhlet extraction process**

4.2.3 Phytochemical screening of O. sanctum

The dehydrated powdered leaves of O. sanctum (150 g each) were subjected to extraction using petroleum ether. The resultant material was subsequently dried and underwent further extraction with ethanol using a repeated procedure. Upon completion of the extraction method, ethanol was entirely evaporated and subsequently treated with methanol to yield a fraction soluble in methanol.

Ultimately, the methanol extract fraction was dried using reduced pressure in order to prepare it for future usage. Photochemical investigations involve doing qualitative chemical screening utilizing various procedures to detect and identify the presence or absence of distinct chemical ingredients. **[13]**

2.3 Formulation development

2.3.1 Formulation of gel base

A sufficient amount of water was used to evenly disperse the gelling chemical. As a plasticiser or humectant, PG-400 was added to dispersion. As extra excipients, methylparaben and propylparaben were added to the mixture while being constantly stirred. The pH of the vehicle in Carbopol gels was neutralised using TEA (triethanolamine). Purified water was eventually used to bring the gel's weight down to 50 grammes. Following that, the mixture was shaken for two hours at 500 revolutions per minute using a propeller. When this homogeneous gel was shaken, there were no air pockets visible. To evaluate the gel's stability and consistency, it was left at room temperature for a whole day.**[14]**

2.3.2 Formulation of hydrogel containing Ocimum Sanctum and Boswellia Serrata

The optimised Carbopol gel and the ethanol extract of Ocimum Sanctum were combined to generate the hydrogel. Then, to create an oil-emulsion, combine the boswellia oil with a moderate emulsifier (Polysorbate 80) in a different container. Slowly add this oil emulsion to the gel that had the ocimum extract before. On the other hand, certain amounts of Boswellia oil will be present in ethanolic extracts of Ocimum Sanctum (1%, 1.5%, 2.5%, 3.5%, and 5%). To the Carbopol dispersion, PG-400, propyl paraben, and methyl paraben were added. To get the required gel consistency and skin pH range of 6.8–7, triethanolamine was gradually added to the mixture. For two hours, a propeller spinning at 500 revolutions per minute was used to stir the mixture. Upon agitation, the gel that was created exhibited a uniform consistency and was free from any presence of air bubbles. The gel that had been made was stored at ambient temperature for duration of 24 hours. [15]

Table 2: formulation of anti-inflammatory gel

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
Ocimum Sanctum (%)	1	2	3	4	5
Boswellia Serrata (oil)	4ml	4ml	4ml	4ml	4ml
Carbapol 940	2%	2%	2%	2%	2%
Propylene glycol 5% (400)	5ml	5ml	5ml	5ml	5ml

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Methyl Paraben	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm
Propyl paraben	4gm	4gm	4gm	4gm	4gm
Triethanolamine	QS	QS	QS	QS	QS
Distilled Water (qs)	100ml	100ml	100ml	100ml	100ml

2.4 Characterization of Prepared Hydrogel

2.4.1 Physical Appearance

The polyherbal hydrogels were evaluated visually for colour, consistency, homogeneity, and odour. The formulations were inspected to confirm uniformity and the absence of aggregates.

2.4.2 pH Measurement

The pH of the formulations was determined using a digital pH meter. One gram of each gel was dispersed in 100 ml of distilled water and kept for 2 h. Measurements were taken in triplicate, and the mean pH value was reported.

2.4.3 Spreadability

Spreadability was assessed using the slip and drag method. Approximately 2 g of gel was placed between two glass slides, one fitted with a hook. A 1 kg weight was placed on the slides for 5 min to obtain a uniform film, and excess gel was removed. An 80 g weight was then applied to the upper slide via a thread attached to the hook. The time required for the slide to move 7.5 cm was recorded, and spreadability was calculated using the formula:

S=M×LTS = \frac{M \times L}{T}S=TM×L

where M = weight tied to the upper slide, L = length moved by the slide, and T = time taken (s). A lower time corresponds to higher spreadability (Gupta et al., [16]).

2.4.4 Homogeneity

Hydrogels were visually inspected for uniform distribution and the absence of clumps or aggregates.

4.4.5 Viscosity

Viscosity was measured using a Brookfield viscometer (spindle no. 7) at 50 rpm and room temperature. The dial readings were multiplied by the appropriate factor provided in the instrument manual to calculate viscosity.

2.5 Drug Content Determination

Two grams of gel were dissolved in 200 ml of ethanol, followed by dilution and filtration. The absorbance of the resulting solutions was measured at 253 nm using a UV–Vis spectrophotometer (Shimadzu UV-1700). Drug content was quantified with reference to a standard calibration curve.

2.6 Stability studies

At 80°C and 40°C, two different storage temperatures, the gel formulation's stability was evaluated. Samples were taken and evaluated for physical characteristics such appearance, homogeneity, pH level, viscosity, phase separation, & drug concentration at intervals of 7, 15, and 30 days. **[18]**

Results and Discussion

3.1 Preformulation studies

Extraction of *Ocimum Sanctum*:

Ocimum sanctum leaves were collected form market and coarse powder of Ocimum sanctum (OSEE) was subjected for the extraction using a Soxhlet apparatus the obtained percent of extract are showing below table.

Table 3: showing the extract yield form the Ocimum powder

Solvent Used	Sample Taken	Extract Yield	Extractive Value %
Ethanol	200g powder	25g	12 % Approx
petroleum ether	150g powder	7.7g	5% Approx
Methanol	150g Powder	15.9g	13 % Approx

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Figure: 3 Extracted products from both bio compound (Tulsi & Indian frankincense resin)

3.1.1 Photochemical studies Ocimum sanctum

Table 3.2 shows the phytochemical analysis of Ocimum sanctum, leaf extracts using two solvents, and aqueous conditions. Following phytochemical screening, a number of bioactive components were found in tulsi leaf extract.

Table 4: Photochemical screening of methanol extract of ocimum scnctum

S.No	Phytochemical	Methanol	Ethanol
1	Cabohydrates	+	+
2	Protein	+	+
3	Phenol	+	-
4	Tannin	+	+
5	Flavonoids	+	+
6	Saponin	+	+

5.1.2 Extractive oil form Indian frankincense resin (Gum)

Table 5: showing the extract yield oil form the frankincense resin

Solvent	Sample Taken	Yield	Extractive Value %	Duration
Distilled Water	450gresins(Gum)	22.5g	5 % Approx	5.5 Hrs

Table 6: physicochemical characteristics Evaluation:

S.No	Properties	Specifications
1	Appearance	Colorless
2	Odor	Fresh, Smooth Musky-Balsamic Aroma
3	Reflective index	1.441- 1.479 at 20°C
4	Specific Gravity (g/ml)	0.841-0.859 at 20°C

3.2 Formulation development

3.2.1 Optimization of Hydrogel galling agent

The concentration of Carbopol-940 was tuned to achieve a gel with the necessary physical properties. The Carbopol gel, when formulated with a concentration of 2%, exhibits favorable physicochemical characteristics for the inclusion of ethanolic extracts derived from Ocimum sanctum and Aloe barbadensis.

3.2.2 Formulation of Hydrogel containing Ocimum *sanctum* and Boswellia Serrata

A hydrogel containing oil from Boswellia serrata and Ocimum sanctum was added to the optimised 2% Carbopol gel basis. To a Carbopol gel basis, different quantities of Ocimum sanctum ethanolic extract—1%, 2%, 3%, 4%, and 5%—were added. In every Carbopol gel basis, the amount of Boswellia serrata was kept constant at 4ml. The created polyherbal gel is shown in the figure below.

3.3 Evaluation of Hydrogel

3.3.1 Physical evaluation of hydrogel

The formulated hydrogel undergoes physical examination for characteristics such as appearance, color, odor, and phase separation. results are presented in Table 3.1 below.

Table 7: Physical evaluation of formulated hydrogel

Parameters	F1	F2	F3	F4	F5
Appearance	Homogeneous	-	-	-	-
Color	Brownish	-	-	-	-

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Odor	Odorless	-	-	-	-
Consistency	Fair	-	-	-	-
Phase separation	**No Separation**	-	-	-	-

(- symbols are showing same results**)**

3.3.2 pH Measurement

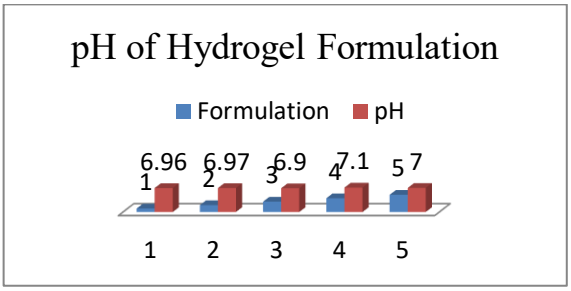

Figure: 4 graphically representation of pH

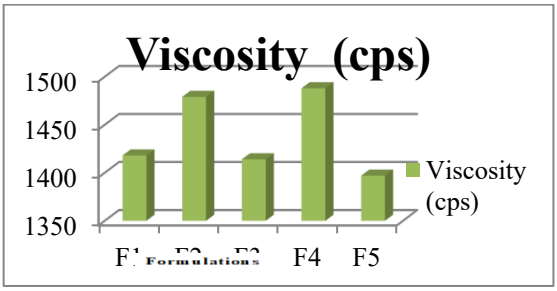

Figure: 5 graphically representation of Viscosity

3.3.3 Spredability

Table 8: Spredability of Hydro-gel

Formulation	**Spredability (gm.cm/sec)**
F1	19.61
F2	19.52
F3	20.84
F4	18.21
F5	22.08

3.3.4 Viscosity

The assessment of the viscosity and rate of drug release from gel is made easier by this rheological property. A Brookfield viscometer with spindle number 62 was used to test the viscosity of the hydrogel that was produced. Table 3.4 presented the findings. The viscosity must be kept below around 15,000 cps in order to provide more appealing aesthetic aspects and make application simpler and more accurate through improved flow and pour-ability.

3.3.5 Drug content

Table 9: Drug content of Optimized hydrogel

Formulation	**Drug content**
F1	94.78± 0.1 %
F2	95.14± 0.2 %
F3	95.75± 0.2 %
F4	94.99± 0.2%
F5	95.90 ± 0.2%

3.3.6 Stability studies

Several formulations were the subject of a stability investigation that lasted one month at 80°C and 40°C. The results of the extraction of specimens at 7, 15, and 30-day intervals are shown in the following table. Over the course of the study, it was observed that all formulations kept at 80°C and 40°C showed homogeneity, with no changes in viscosity, colour, or odour. The drug concentration, viscosity (measured in centipoises), and spreadability have all slightly changed.

Table 10: Accelerated Stability studies

Parameter	**Storage condition** (80^0C, 40^0C) 30days					
	7 days		**15 days**		**30 days**	
	80	**40**	**80**	**40**	**80**	**40**

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Color	Brownish	-	-	-	-	-
Odor	Odorless	-	-	-	-	-
Viscosity(cp)	1397±0.1	1386±01	1397±02	1387±02	1397±01	1384±01
Spreadability (gm.cm/sec)	22.01	21.41	21.83	21.10	20.65	20.78
Drug Content	95.89 ± 0.2%	95.87 ± 0.2%	95.41 ± 0.2%	95.09 ± 0.2%	95.00 ± 0.2%	94.98 ± 0.1%
Phase separation	No separation	-	-	-	-	-

Note: (- Indicate No separation**)**

Conclusion:

In order to improve the therapeutic effectiveness of medications applied topically, gel-based drug delivery techniques have become more and more popular in recent years. Indian frankincense and Ocimum sanctum are both known to have anti-inflammatory properties. Ocimum sanctum has antioxidative, antifungal, and antibacterial properties. For the hydrogel containing Ocimum sanctum, the topical route was used to maximise concentration at the intended location of action and avoid gastrointestinal tract discomfort. The use of penetration enhancers is necessary for the transdermal delivery of the medication in order to get beyond the restrictions caused by the skin's barrier properties. The inclusion of flavonoids, tannin, and saponin in both plant extracts may be the reason for the well-established anti-inflammatory effects of Ocimum sanctum Indian frankincense extracts.

Finally, we deduce that for ages, the resin of Ocimum sanctum and Boswellia species—commonly referred to as "frankincense" or "olibanum"—has been used as incense in religious & cultural rituals. It is widely known to provide therapeutic benefits, especially when it comes to treating inflammatory diseases, some types of cancer, wound healing, and having antibacterial qualities. However, Boswellia and tulsi have not been well studied, despite their historical, religious, cultural, and therapeutic value. As a result, there is a discrepancy between traditional uses of the resin & existing scientific data. For these reasons, the old medical system is still widely used.

The focus on using plant materials as a medicinal source for a wide range of human ailments has increased due to factors such as rapid population growth, a lack of branded medications, high treatment costs, the negative side effects of many allopathic drugs, & rising resistance to current drugs for infectious diseases. However, a steady and continuous supply of these source materials is frequently difficult to achieve because of things like shifting environmental conditions, regional cultural customs, diverse geographic dispersion, growing labour expenses, poor plant stock selection, and unethical business practices in the pharmaceutical sector.

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