Formulation and Evaluation of Basil Seeds Oil Based Wound Healing Gel

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Abstract*— The increasing interest in natural and sustainable therapeutic agents has driven research into plant-based formulations for wound care. This study focuses on the development and evaluation of a basil seed oil-based wound healing gel, leveraging the known bioactive properties of basil seed oil without the use of animal models. Basil seed oil, rich in anti-inflammatory, antimicrobial, and antioxidant compounds, was incorporated into a gel matrix using biocompatible polymers such as Carbopol and konjac gum to create a stable and effective topical gel.The formulation process involved optimizing various parameters, including oil concentration, polymer type, and cross-linking agents, to achieve a gel with desirable physicochemical characteristics. Key properties such as pH, viscosity, spreadability, and rheology were meticulously analyzed to ensure the gel's stability and ease of application. The antimicrobial efficacy of the basil seed oil gel was tested against a spectrum of wound-associated pathogens, including Staphylococcus aureus, Escherichia coli, and Candida albicans, using agar diffusion and minimum inhibitory concentration (MIC) methods. This study underscores the promising potential of basil seed oil in advancing wound care and promoting natural healing processes.

Index Terms- Therapeutic Agent, Basil Seeds Oil, Anti-Microbial, Carbopol, Konjac Gum, Pathogens, Wound Healing Gel.

I. INTRODUCTION

A wound is an injury to living tissue, typically involving a break or disruption in the skin or other body membranes. Wounds can be caused by various factors, including physical trauma, surgery, burns, or diseases that compromise the integrity of the skin and underlying tissues [1]. Wound healing is a complex physiological process crucial for the restoration of tissue integrity and function. Wound healing process obtained through four highly programmed stages: hemostasis, inflammation, proliferation and remodeling. For a wound to be healed effectively, all these stages must occur in the appropriate sequence and time frame [2].

1 Types of Skin Wounds [3]

1.1 Acute Wounds

1. Abrasions
- Description: Superficial wounds caused by friction.
- Characteristics: Scraped skin, minor bleeding.
- Example: Skinned knee.

2. Lacerations
- Description: Cuts or tears in the skin.
- Characteristics: Irregular edges, varying depth, may bleed heavily.
- Example: Cut from a sharp object like a knife.

3. Punctures
- Description: Small, deep holes in the skin.
- Characteristics: Minimal surface damage, high infection risk.
- Example: Stepping on a nail.

4. Burns
- First-Degree: Affect only the outer layer (red, painful).
- Second-Degree: Affect outer and underlying skin layers (blistering).
- Third-Degree: Extend into deeper tissues (white or charred skin, may be numb).

1.2 Chronic Wounds [3]

1. Pressure Ulcers (Bedsores)
- Description: Caused by prolonged pressure on the skin.
- Characteristics: Red, sore areas that can develop into deep wounds.
- Example: Bedridden patients.

2. Diabetic Ulcers
- Description: Wounds that develop due to poor circulation and nerve damage in diabetics.
- Characteristics: Commonly on the feet, slow to heal.
- Example: Foot ulcers in diabetic patients.

3. Venous Ulcers
- Description: Result from poor blood flow in the veins.
- Characteristics: Often occur on the legs, swollen and painful.
- Example: Sores on the lower legs.

In contemporary conventional therapy, wound healing typically involves the systemic administration of antibiotics and the topical application of antiseptics. These agents primarily function to maintain sterility in the wound area, preventing further infection but not actively participating in the physiological repair process of wound healing [4]. However, nature provides a wealth of herbs that can aid in the secondary intention healing process. The ongoing use of certain herbs and chemical agents can enhance the synthesis of fibro genetic and collagen, leading to faster wound healing. Despite advancements in medical science, effective wound management remains a significant challenge, particularly due to the emergence of antibiotic-resistant pathogens and the limited availability of affordable treatment options [5]. In recent years, there has been growing interest in exploring natural remedies, such as plant-derived oils, for their therapeutic potential in wound care.

It is generally accepted that plants and their products possess immense potential for the treatment of wounds and cutaneous inflammations. The demand for herbal formulations is increasing in both developed as well as developing countries, as they are widely available and safe to use and better tolerated than allopathic drugs, promoting repair mechanisms in a natural way, herbal-derived dressings represent a low-cost alternative for wound healing, in the context of bacterial resistance [6]. Now a days antibiotic-loaded wound dressings are often associated with the excess of synthetic drugs and the occurrence of bacterial resistance, as well as toxicity and de-pigmentation of the wound area. According to the WHO, it is estimated that 80% of the world population prefers herbal-based treatments for dermatological diseases, cancer, diabetes and other diseases. [6,7].

It is therefore essential to identify and utilize such herbs, shifting towards herbal therapy for more efficient wound healing outcomes. Topical drug administration, a localized drug delivery system, can be applied anywhere on the body through ophthalmic, rectal, vaginal, and skin routes [8]. For treating dermatological diseases and skin care, a variety of formulations available today ranging from solids to semisolids and liquid preparations, are available for both clinicians and patients.

Among semisolid preparations, transdermal gels have gained popularity in both cosmetics and pharmaceuticals. Transdermal application of gels at pathological sites offers significant advantages, providing faster drug release directly to the site of action, regardless of the drug's water solubility, compared to creams and ointments [9].

Topical formulations suitable for wound healing and reduce pain. They are easy to formulate and inexpensive. It would also be ideal for the formula to possess antimicrobial properties, to be easily removed when necessary and to be able to absorb the exudate from the wound [10,11]. Of the many existing topical formulas, gels possess most of the above properties. Other important features of gels include the promotion of wound contraction and allowing a flow of oxygen to support the respiratory activity of the epithelial cells. Clinical studies have confirmed that moist conditions lead to faster wound contraction and can accelerate wound healing by up to 50%[12].

II. GEL

A gel is a semi solid substance that has properties between those of liquids and solids. It typically consists of a liquid dispersed in a solid matrix, forming a jelly like consistency. A gel is a semi solid that can have properties ranging from soft and weak to hard and tough[13]. Gels are defined as a cross linked system, which exhibits no flow when in the steady state, although the liquid phase may still diffuse through this system. A gel has been defined phenomenologically as a soft, solid or solid-like material consisting of two or more components, one of which is a liquid, present in substantial quantity[14]. When a warm gelatin solution is cooled, semisolid mass forms. This process of gel formation is called gelation.

Types Of Gels[15]: Elastic gels, non-elastic gels, Controlled release gels, Organogels, Hydrogels, Xerogels.

Properties Of Gels: Hydration, Swelling, Syneresis, Thixotropy.

Fig 1: Gel

2.1 Advantages of gels [16,17]

1. Moist Environment: Gel provide moist environment, which simplifies common healing phases such as granulation, epidermal repair and removal of excess dead tissue.
2. Pain Relief: The cool sensation provided by gels offers relief from pain for at least six hours.
3. Biocompatibility: Gels are generally biocompatible, meaning they are well-tolerated by the body and do not cause adverse reactions or inflammation.
4. Controlled Drug Delivery: Some gels can be designed to release drugs or growth factors gradually over time, aiding in wound healing and tissue regeneration.
5. Easy Application: Gels are easy to apply and can conform to irregular wound shapes, ensuring good contact with the wound bed.

2.2 Disadvantages of gels [15,17]

1. Limited Fluid absorption: Gels cannot absorb large amounts of fluid, making them unsuitable for very wet wounds. Excessive moisture could lead to maceration and infection.
2. Low Mechanical Strength: Gels have low mechanical strength, which makes them prone to tearing easily. This can be problematic for patients who need to change their own dressings.
3. Swelling and De-swelling: Gels can swell significantly when exposed to water or body fluids. While this property is useful for drug delivery or wound healing, it can also lead to discomfort or pressure on surrounding tissues.
4. Biodegradation rate: The rate of biodegradation varies among different gel compositions. Some gels degrade too quickly, while others may remain intact for extended periods. Finding the right balance is crucial for their intended application.
5. Limited Drug Loading Capacity: Gels have a finite capacity for incorporating drugs or bioactive molecules. This limitation affects their use in sustained drug release systems.

III. BASIL ESSENTIAL OILS

Basil essential oils (BEOs) are natural volatile compounds contains various chemical constituents produced through plant secondary metabolism. These oils are widely used in industries such as pharmaceuticals, cosmetics, perfumery, dental and oral products, aromatherapy, and food [18]. Traditionally, basil has been employed for its positive effects against fever, cough, flu, asthma, and bronchitis. Within the Balkan diet, basil is recognized for its radio-protective, anti-inflammatory, anti-stress, anti-diabetic, and anti-pyretic properties [19]. *Ocimum* species possess an abundance of phenolic acids, also volatile oils.

BEOs are known for their antimicrobial, antibacterial, antifungal, and antioxidant properties. They serve as natural preservatives for fresh produce post-harvest, leaving no residual effects. Furthermore, BEOs are integral to biopesticides, exhibiting repellent, larvicidal, and insecticidal properties [20].

Fig 2: Basil oil

IV. LITERATURE REVIEW

1. Barkat Ali Khan (2020) et al, this study examines the wound healing potential of an emulgel containing

Ocimum basilicum extract. Evaluation through various tests including FTIR analysis and in vitro drug release confirms the compatibility of the extract with selected polymers. In vivo studies on rabbits reveal significant wound contraction and histopathological improvements, to a commercial Silver Sulfadiazine cream. These findings underscore the emulgel's promise as a novel therapeutic avenue for wound healing applications.

2. Zoran S. (2021) et al, this article highlights about efficacy of an emulgel enriched with *Ocimum basilicum* extract for wound healing purposes. By conducting thorough analyses like FTIR and in vitro drug release assessments, the study validates the compatibility of OB extract with selected polymers and excipients. Through in vivo trials on rabbits, the emulgel showcases notable wound contraction and histopathological enhancements. These results underline the emulgel's potential as a compelling therapeutic option for wound care, signaling promising prospects for future clinical adoption.

3. Rajesh k. Joshi (2014) et al, article investigates the potential of *Ocimum basilicum* extract in wound healing applications. Through comprehensive assessments, including histological analysis and wound closure measurements, the study demonstrates the extract's efficacy in promoting wound healing in animal models. The results reveal significant improvements in wound contraction and tissue regeneration compared to control groups. These findings underscore OB extract's promising therapeutic value in wound care, highlighting its potential for further clinical exploration and development.

4. Ina Andreea Antonescu (Mintas) (2021) et al, investigates the wound healing potential of a newly developed hydrogel incorporating *Ocimum basilicum extract*. Utilizing a range of analyses such as histological examination and wound closure assessments, the study demonstrates the hydrogel's efficacy in enhancing wound healing in animal models. Notable improvements in wound contraction and tissue regeneration are observed compared to control groups. These findings underscore the promising therapeutic application of the OB extract-infused hydrogel in wound management, suggesting avenues for future clinical exploration and development.

5. Pongsak Rattanachaikunsopon (2010) et al, the research explores the antimicrobial properties of basil (*Ocimum basilicum*) oil against Salmonella Enteritidis. Their findings indicate that basil oil exhibited potent antimicrobial activity against both reference and clinical strains of S. Enteritidis, with significant inhibitory effects observed in vitro and in food matrices like nham, a fermented pork sausage. Gas chromatography/mass spectrometry analysis identified key constituents of basil oil, such as linalool, 1,8-cineole, and eugenol, contributing to its antimicrobial efficacy. The study underscores the potential of basil oil as a natural antimicrobial agent for controlling S. Enteritidis in food products

6. Monika Sienkiewicz (2013) et al, antimicrobial activity of basil (*Ocimum basilicum*) has been extensively studied, revealing that its essential oils and extracts exhibit significant inhibitory effects against a range of bacteria, fungi, and viruses. The primary active compounds, including linalool, eugenol, and methyl chavicol, contribute to its efficacy. Studies show that basil essential oil is effective against common pathogens like Staphylococcus aureus, Escherichia coli, and Candida species. This suggests basil's potential as a natural preservative and a complementary treatment for infections.

V. MATERIALS AND METHODS

5.1 Extraction of *Ocimum basilicum* oil [21]

The hydrodistillation process for extracting oil from basil seeds starts by cleaning and drying the seeds. These seeds are placed in a distillation flask with water, and the mixture is heated to boiling. The heat causes the water to vaporize along with the essential oils from the seeds, creating steam that carries the oils. This steam passes through a condenser, cooling down and condensing back into a liquid form. The condensed liquid, a mixture of water and essential oil, is collected in a receiver. Due to their immiscibility, the essential oil can be separated from the water using a separatory funnel. The essential oil is then further dried using anhydrous sodium sulfate to remove any residual water. Finally, the purified oil is filtered and stored in a dark glass bottle to protect it from light and ensure its longevity.

Fig 3: Hydrodistillation

5.2 Preparation of gel

The gel is made by dispersing carbopol or konjac gum (in case of konjac gum as a gelling agent) in water under stirring to form uniform gel base. Then, glycerin is added to this solution as a humectant to retain moisture in the gel. Afterward, basil oil, known for its antimicrobial properties, is incorporated into the carbopol gel along with tween 80 as an emulsifier to ensure uniform dispersion. Triethanolamine (TEA) is added dropwise to adjust the pH and to neutralize the carbopol gel. Methyl paraben may also be added as a preservative to prevent microbial growth. The mixture is then homogenized to achieve a smooth and homogeneous gel formulation. Finally, the gel is left to hydrate and thicken, resulting in a stable gel with basil oil imparting antimicrobial activity.

Fig 4: Magnetic stirrer

Table 1: Formulation chart for preparation of gel

S. No	Ingredients (%w/w)	F1	F2	F3	F4	F5	F6
1.	Basil oil (ml)	1	1	1	1	1	1
2.	Konjac gum (%)	0.25	0.5	0.75	-	-	-
3.	Carbopol (%)	-	-	-	0.25	0.5	0.75
4.	Glycerine (%)	2	2	2	2	2	2
5.	Triethanolamine (drops)	1	1	1	1	1	1
6.	Methyl paraben (%)	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
7.	Tween 80 (%)	1	1	1	1	1	1
8.	Distilled water (%)	50	50	50	50	50	50

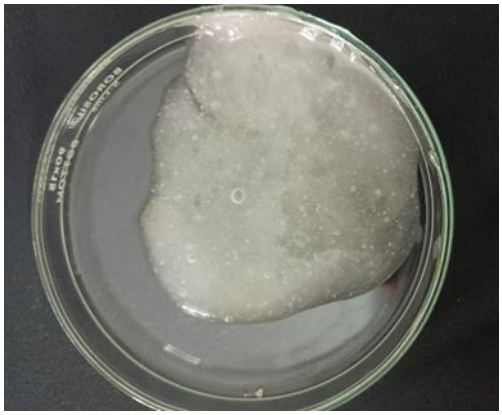

Fig 5: Formulation with Konjac gum

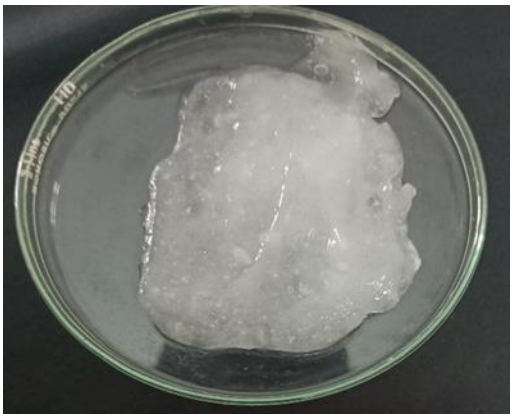

Fig 6: Formulation with Carbopol

VI. EVALUATION TESTS

6.1 Organoleptic evaluation [22]

- Color
- Odour
- Texture
- State

6.2 pH test: At constant temperature, the pH of the formulation was determined using digital pH meter.

Fig 7: Digital pH Meter

6.3. Transparency test: Transparency of formulation was assessed manually.

6.4. Washability: After applying a tiny amount of gel to the hand, it was washed with tap water, observe its wash ability.

6.5. Spreadability [23]: The spread ability was measured by the time it took two slides to slip away from the gel, which was placed in between those slides, under a force. Two sets of standardized glass slides were taken. The gel mixture was then placed on a slide of appropriate size. The formulation was then placed on top of second slide. The gel between the two slides was then pushed uniformly to form a thin layer when a weight or specified load was placed on the upper slide. The weight was then removed and any excess formulation stuck on the slides was scraped away. The length and time it took for the upper slide to fall off was noted.

Spreadability was calculated by using the following formula,

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

Where, S = Spreadability

M= Weight tied to upper slide

L=Length of the glass

T=Time

6.6. Irritancy test: Gel is applied to the dorsal side of the hand and left it overnight. After 6 hours irritability and allergic reactions are assessed.

6.7. Phase Separation [24]: The prepared gel was maintained at a temperature of 25-100 °C, away from light, in a sealed container. Then the next 30 days, phase separation was monitored for every 24 hours. The phase separation was examined and confirmed for any changes.

6.8. Extrudability: Extrudability test was carried out by using Pfizer hardness tester. 15gm of gel was filled in collapsible aluminium tube. The plunger was adjusted to hold the tube properly the pressure of 1/2kg/cm2 was applied for 30 sec. The quantity of the gel extruded was weighed. The procedure was repeated at three different places of the tube.

6.9. Viscosity [23]

Brookfield digital viscometer was used to measure the viscosity of gel formulations. The spindle no. 6 was dipped in gel sample rotated at 10 rpm for 15 min. The reading in triplicate was noted. Viscosity in centipoise (cp) was measured.

Fig 8: Brook field viscometer

6.10. Preliminary phytochemical analysis

Preliminary analysis was carried out to identify the useful constituents like phenols, flavonoids, fatty acids, tannins[13,14].

1. Phenols: Around 20-30%
2. Fatty acids: Around 30-40%
3. Flavonoids: Less than 5%
4. Terpenes: Around 5-10%

6.10.1. Determination of total phenolic content[22]

Ferric chloride test: Phenols react with ferric chloride to form colored complex. This test can indicate the presence of phenolic compounds based on color change.

6.10.2. Determination of flavonoids

TLC plate was developed by using solvents ethyl acetate: methanol: water in the ratio of 7:2:1. Visualization of spots was done by spraying aluminum chloride and observed under UV spectrophotometer.

6.10.3. Determination of terpenes

TLC plate was developed by using solvents hexane and ethyl acetate in the ration of 7:3. Visualization of spots was done by spraying sulfuric acid and observed under UV spectrophotometer.

6.10.4. Determination of fatty acids

TLC plate was developed by using solvents hexane and ethyl acetate in the ratio of 9:1. Visualization of spots was done by spraying iodine and observed under UV spectrophotometer.

6.11. Antimicrobial activity for the optimized formulation

The anti-microbial activity of each formulation was carried out using the technique of zone of inhibition, employing Escherichia coli and staphylococcus aureus as test organisms.

6.11.1. Preparation of nutrient broth medium[25]

The ingredients such as nutrient agar is used to prepare medium for bacteria. Sub agarose, dextrose and peptone were dissolved in water with the aid of heat to prepare medium for Candida albicans. The pH was adjusted to 7.1± 0.1. This medium was then autoclaved at a temperature of 121°C and 15 lb pressure for 30 minutes. Later, subculture E. coli and S. aureus, Candida albicans were introduced into every agar plate, employing strict aseptic conditions (under laminar flow). The sterilized media were carefully transferred into sterilized Petri dish and allowed to solidify. Then bore diameter of 4mm was made in the nutrient culture media. Equal quantity of individual semisolid formulations was added carefully into the bore made and these petri dishes were incubated at temperature of 37 °C for 48 hours for the growth of the microorganisms to take place. Later, the zone of inhibition produced by the formulations towards E. coli and S. aureus and candida albicans organisms were measured individually in all the Petri dish and photographed.

Table 2: Ingredients used to prepare medium for fungi

S. No	Ingredients	Quantity(25ml)
1	Sub agarose	1.2
2	Peptone	0.4
3	Dextrose	0.25
4	Water	qs to 25ml

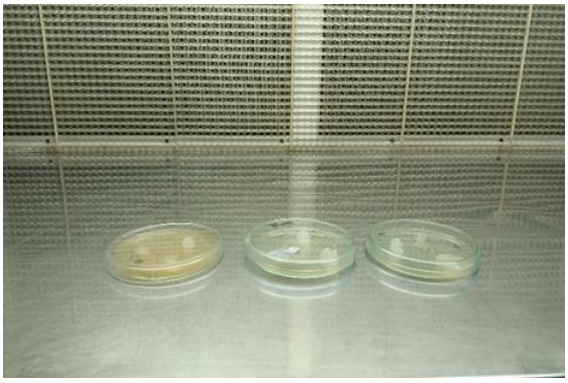

Fig 9: Laminar air flow

VII. RESULTS

Table 3: Organoleptic evaluation

Formulation code	Color	Odour	Texture	State
F1	Transparent	Pleasant	Smooth	Semi solid
F2	Transparent	Pleasant	Smooth	Semi solid
F3	Transparent	Pleasant	Smooth	Semi solid
F4	Transparent	Pleasant	Smooth	Semi solid
F5	Transparent	Pleasant	Smooth	Semi solid

F5	Transparent	Pleasant	Smooth	Semi solid

Table 4: Physicochemical Assessmet

S.No	Formulation code	pH
1.	F1	6.6
2.	F2	6.5
3.	F3	6.6
4.	F4	6.8
5.	F5	6.8
6.	F6	6.5

Table 5: Washability

S.No	Formulation code	Washability
1.	F1	Easily washable
2.	F2	Easily washable
3.	F3	Easily washable
4.	F4	Easily washable
5.	F5	Easily washable
6.	F6	Easily washable

Table 6: Spreadability

S.No	Formulation code	Time(sec)	Spreadability (cm/g)
1.	F1	8	4
2.	F2	8	4
3.	F3	7	3.5
4.	F4	9	5
5.	F5	5	6
6.	F6	4.5	7

Table 7: Irritancy test

S.No	Formulation code	Irritatancy
1.	F1	No irritation
2.	F2	No irritation
3.	F3	No irritation
4.	F4	No irritation
5.	F5	No irritation
6.	F6	No irritation

Table 8: Phase separation

S.No	Formulation code	Phase separation
1.	F1	No
2.	F2	No
3.	F3	No
4.	F4	No
5.	F5	No
6.	F6	No

Table 9: Extrudability

S.No	Formulation code	Extrudability
1.	F1	Easily extrudable
2.	F2	Hardly extrudable
3.	F3	Hardly extrudable
4.	F4	Easily extrudable
5.	F5	Easily extrudable
6.	F6	Easily extrudable

Table 10: Viscosity

S.No	Formulation code	Viscosity (cps)
1.	F1	4211
2.	F2	4205
3.	F3	4523
4.	F4	4251
5.	F5	4525
6.	F6	4650

Table 11: Phytochemical tests

S.No	Plant constituent	Result
1.	Phenols	Positive
2.	Flavonoids	Positive
3.	Terpenes	Positive
4.	Fatty acids	Positive

Fig 10: TLC of fatty acids

Fig 11: TLC of terpenes

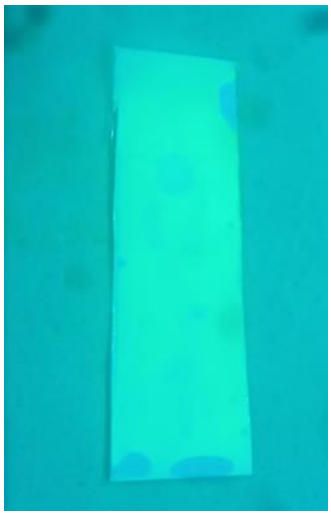

Fig 12: TLC of flavonoids

Fig 13: Ferric chloride test for phenols

Antimicrobial activity

Table 12: Microbiological Evaluation of Gel

S. No	Name of the Microorganism	Result
1.	Staphylococcus Aureus	Negative
2.	E-coli	Negative
3.	Candida Albicans	Negative

VIII. SUMMARY

The study focused on formulating and evaluating a basil seed oil wound healing gel with a comprehensive approach. Initial assessments included checking color, odor, texture, and consistency to ensure the gel met quality standards. Practical tests such as pH measurement, transparency evaluation, and washability demonstrated its physical properties and suitability for use.

Performance evaluations, including spreadability and extrudability tests, provided insights into how easily the gel could be applied. Viscosity measurements indicated its flow characteristics, essential for practical application. Phytochemical analysis identified beneficial compounds like phenols, fatty acids, flavonoids, and terpenes, suggesting potential therapeutic benefits.

Antimicrobial tests against Escherichia coli and Staphylococcus aureus highlighted the gel's ability to inhibit bacterial growth, showing promise for managing wound infections. Overall, the study covered formulation, physical characterization, chemical analysis, and initial efficacy testing. Overall, the study provided insights into the gel's properties and its potential application in wound care.

CONCLUSION

Study focused on the comprehensive formulation and evaluation of a basil seed oil gel intended for wound healing applications. Through rigorous testing, we ensured the gel met essential quality standards, including organoleptic assessments for color, odor, texture, and consistency. Practical tests such as pH measurement, transparency assessment, and washability confirmed its physical characteristics and suitability for use in clinical settings.

Performance evaluations, including spreadability, extrudability, and viscosity measurements, provided valuable insights into the gel's practical application and handling. Phytochemical analysis revealed the presence of bioactive compounds like phenols, fatty acids, flavonoids, and terpenes, indicating potential therapeutic benefits for wound healing.

Crucially, antimicrobial testing against common pathogens such as Escherichia coli and Staphylococcus aureus demonstrated the gel's ability to inhibit bacterial growth, suggesting its potential effectiveness in preventing and treating wound infections. Based on the findings from our study, as we compared the efficacy of synthetic and natural gelling agent in the formulation. Our findings indicate that the synthetic gelling agent outperforms the natural gelling agent in terms of over effectiveness. It provided a more uniform gel texture which is crucial for consistent application and absorption of active ingredients. F6 formulation of basil seed oil gel emerges as the optimal choice for wound healing applications through comprehensive evaluation and comparison with other formulations

Overall, our study underscores the promising role of basil seed oil gel in wound care, emphasizing its formulation robustness, physical properties, chemical composition, and initial efficacy against microbial threats. Further research and clinical validation will validate its clinical benefits and cement its role in modern wound management practices.

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