WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 19, 920-938. Research Article ISSN 2277–7105

FORMULATION DEVELOPMENT AND EVALUATION OF HERBAL OINTMENT CONTAINING *NIGELLA SATIVA* AND *ALTHAEA OFFICINALIS* EXTRACTS FOR WOUND HEALING

Abdi Ahmed Ebrahim[1]* and Dr. Vishvesh Kanabar[2]

Department of Pharmaceutics, School of Pharmacy, RK University, Rajkot, Gujarat, India.

Article Received on
11 Aug. 2024,
Revised on 01 Sept. 2024,
Accepted on 22 Sept. 2024
DOI: 10.20959/wjpr202419-34032

***Corresponding Author**
Abdi Ahmed Ebrahim
Department of Pharmaceutics, School of Pharmacy, RK University, Rajkot, Gujarat, India.

ABSTRACT

Background: The recovery of wounded tissues depends on the wound healing process, which calls for the use of efficient formulations to speed up the healing process. In our research, we incorporated herbal materials with established therapeutic qualities into topical formulations as ointment dosage form. *Nigella sativa* and *Althaea officinalis* have emerged as viable options for wound treatment. Although each is known to have specific therapeutic benefits, their ability to work in synergy in a combination formulation has not yet been investigated. With an emphasis on formulation development and evaluation of an ointment incorporating *Nigella sativa* (Black seeds) and *Althaea officinalis* (Marshmallow) extracts, our project seeks to provide important insights into herbal-based therapies for wound healing. **Objective:** To incorporate the beneficial properties of *Nigella sativa* and *Althaea officinalis* as ointment dosage form to advance natural-based therapies for wound healing. **Materials and Methods**: Physicochemical analysis of drugs was performed. The ointment base was prepared by melting hard paraffin and cetostearyl alcohol on a water bath, followed by the addition of wool fat and white soft paraffin with gentle stirring for homogeneous mixing. The herbal ointment was then formulated by weighing *Nigella sativa* and *Althaea officinalis* extracts, separately measuring the ointment base, and triturating the active ingredients with portions of the base in a mortar using a pestle until a uniform distribution of the active ingredients was achieved. The formulated ointment was evaluated for various parameters. **Results and Discussion:** The prepared ointment had a smooth texture, a grayish-black color, a semi-solid consistency, and a characteristics odour. The pH values (i.e. 6.16±0.12-6.83±0.20) showed favorability of the formulation for topical applications. Spreadability results (i.e. 7.74±0.80-29.12±1.30 gm.cm/sec) demonstrated good spreadability. Extrudability results (i.e. 80.2±1.27%-95.8±2.10%) indicated ease of application. Viscosity measurements values indicated that as the concentration of hard paraffin decrease from F1 to F5, there is a corresponding decrease in viscosity. Stability study revealed that F2, F3, F4 and F5 were remained stable across various temperature conditions (i.e. 5±3 °C, 25±2 ℃, 37±2 ℃) over a four-week period. **Conclusion:** The project explored on the herbal-based wound therapy by using combined potential of Nigella sativa and Althaea officinalis extracts. The formulated ointment exhibited favorable physical properties, pH levels, spreadability, extrudability, viscosity, and stability. Based on the observed results, F5 appears to be the best ointment formulation. The results of this study pave the way for advancements in herbal-based therapies for wound healing.

KEYWORDS: Herbal Ointment, Nigella Sativa (Black Seed), Althaea Officinalis (Marshmallow), Wound Healing.

INTRODUCTION

Wounds are injuries that result in a break or opening in the skin or other tissues of the body. In pathology, a wound is acute injury that damages the epidermis of skin. Numerous factors, such as trauma, illnesses, accidents, or other skin problems like psoriasis or eczema that may develop in the area, can cause the wounds.[1]

The wound healing process is the response of the organism to an injury.[2] The process typically involves a series of well-coordinated events that aim to restore the structure and function of the injured tissue.[3] Hemostasis, inflammatory phase, proliferative phase, and maturation (remodeling) phase are the four steps of the wound healing process.[4] As a fundamental aspect of healthcare, the quest for effective and innovative formulations to expedite wound recovery continues to be a focal point of research.

Nigella sativa and Althaea officinalis stand out among the vast array of botanicals known for their therapeutic potential in the context of wound healing.[5][6] Nigella sativa (Black seeds) have been recognized for wound healing due to their anti-inflammatory, antimicrobial, and antioxidant properties.[7-9] Similarly, Althaea officinalis (Marshmallow) has a long-standing history in wound healing for its antibacterial, anti-inflammatory, mucilaginous and soothing attributes.[10-12] While both these botanicals have been individually explored for their medicinal applications, their synergistic potential in a combined formulation remains unexplored area.

An ointment is a semisolid pharmaceutical dosage that is typically applied topically to the skin or mucous membranes.[12] All ointments consist of a base which acts as a carrier for active ingredients.[13] Ointments provide a valuable advantage in wound care through their site-specific application directly to the affected area. It creates a protective barrier over the wound, shielding it from contaminants and promoting a favorable healing environment.

The primary objective of this study was to formulate herbal ointment containing Nigella sativa and Althaea officinalis extracts with a suitable base, to incorporate the beneficial properties of Nigella sativa and Althaea officinalis for advancing natural-based therapies in wound healing, and to evaluate formulated ointment.

Table 1: Stages of Wound Healing.

Sr. No.	Stage	Description	Time frame
1	Hemostasis	• Involves the immediate response to injury, where blood vessels constrict to reduce blood loss. • The primary aim of this stage is to halt any bleeding. This is achieved as your body triggers its blood coagulation mechanism.	Immediately after injury
2	Inflammatory Phase	• Inflammation is a crucial part of the healing process. • White blood cells, especially neutrophils and macrophages, migrate to the wound site to remove debris, bacteria, and damaged tissue. • The inflammatory response helps create a clean environment for tissue repair and sets the stage for the next phase.	Hours to days after injury
3	Proliferative Phase	• During this stage, new tissue is formed to replace the damaged or lost tissue. • Fibroblasts produce collagen, a structural protein that provides strength to the wound. • Blood vessels also regenerate to supply the developing tissue. • Epithelial cells at the wound edges start to migrate and cover the wound surface.	Days to weeks after injury.
4	Maturation (Remodeling) Phase	• In this final phase, the collagen fibers undergo reorganization and remodeling. • The initially deposited collagen is replaced with stronger and more organized collagen tissue. • The scar tissue formed during the proliferative phase undergoes changes to improve its strength and function. While the wound may appear healed externally, the remodeling process continues, and the scar tissue gradually matures.	Weeks to months after injury.

Drugs profile

1. *Nigella sativa* (Black seed)

- Synonyms: Black cumin, Black caraway, Nigella, Kalonji, Charnushka
- Biological source: *Nigella sativa*
- Family: Ranunculaceae
- Chemical constituents: Fixed oils (26% to 38%), proteins, alkaloids, saponins (melanin), and essential oil (0.4% to 2.5%). The major component of the volatile essential oil is **thymoquinone** (28% to 57%). Thymoquinone, thymohydroquinone, dithymoquinone, and thymol are considered as the main active constituents.

Figure 1: Nigella sativa powdered seed.

The following describes the properties of black seeds in relation to wound healing:

- Anti-Inflammatory Effects: Black seeds contain chemicals with anti-inflammatory capabilities, including thymoquinone. The healing process might go more easily if the area surrounding the wound is less inflamed.
- Antioxidant potential: Black seeds' antioxidant qualities can shield cells from oxidative stress, which can impede the healing of wounds. Antioxidants support the regeneration and repair of cells.
- Antimicrobial Activity: Black seeds' antimicrobial qualities that can aid in preventing wound infections and encouraging sterile healing conditions.

2. *Althaea officinalis* (Marshmallow)

- Synonyms: Althea root, A. officinalis
- Biological source: *Althaea officinalis*
- Family: Malvaceae Juss.

- Chemical constituents: Starch (25–35%), pectins (11%), saccharose (10%), **mucilage** (5%), flavonoids, caffeic acid, p-coumaric acid, isoquercitrin, coumarins, phytosterols, tannins, etc., as well as many amino acids (Gudej, 1991; Bradley(ed.), 1992).

Figure 2: Marshmallow Root Powder.

The following describes the mechanisms that make marshmallow useful for wound healing:

- Moisture Retention: Using formulations containing marshmallow on wounds can help retain moisture in the surrounding area. Moist wound healing speeds up the healing process and lessens scarring.
- Calming Irritation: Marshmallow's mucilage helps relax and soothe inflamed skin surrounding wounds. This is especially helpful for potentially sensitive or irritated wounds.
- Protection: Marshmallow mucilage's gel-like consistency can form a barrier to shield the wound from outside contaminants and encourage a sterile healing environment.
- Pain Reduction: Marshmallow's calming qualities can ease the discomfort and pain brought on by some kinds of wounds.

Introduction to Ointment

An ointment is a semisolid preparation that is typically applied commonly to the skin surfaces. It is pharmaceutical dosage form that consists of a base combined with active ingredients that provide therapeutic effects.

Characteristics of an ideal ointment[14]

- It should demonstrate both chemical and physical stability.
- Active ingredient should be uniformly distributed in ointment base.
- The ointment should be sooth and free from grittiness
- The base of ointment should possess no therapeutic action.

Advantages of ointment[15]

- Targeted application of medication to the affected site.
- Convenient for unconscious patients.
- They avoid first pass metabolism of drug.
- They are suitable dosage forms for bitter taste drugs.
- They are chemically more stable and easy to handle than liquid dosage forms.

MATERIALS AND METHODS

Materials

The black seed used in this research was sourced from Awra Amba, Ethiopia. The marshmallow root powder utilized in this study was purchased from VedoMax Herbals. Both active ingredients are identified by the expert of RK University. Other ingredients vital to our formulation were sourced from Pharmaceutics lab, School of Pharmacy at R.K University.

Table 2: List of Material.

Materials	Manufacturer/ Distributor	Description
Black seed *(Nigella sativa)*	Awra Amba	Drug
Marshmallow *(Althaea officinalis)*	Vora kadar & sons	Drug
Wool fat	Astron Chemicals	Emollient
Cetostearyl alcohol	Astron Chemicals	Emulsifier
Hard paraffin	Molychem	Thickener
White soft paraffin	Astron Chemicals	Base

Table 3: List of Equipment.

Equipment	Manufacturer	Description
Digital weighing scale	Swisser instruments	To measure the precise quantities of the active ingredients and the base.
Digital pH meter	Spectronics, India	For determining pH of formulation
Brookfield Viscometer	Brookfield Engineering, USA	For determining viscosity of formulation
Hot Air Oven	LABPRO®, India	For drying and determining LOD of formulation
Water bath	Laboratory deal, India	For temperature control and homogeneous Mixing

Physicochemical analysis of *Althaea officinalis* and *Nigella sativa*

1. Determination of total ash[16]

Weighed 3 grams of dried powdered drug in a silica crucible.

⇩

Incinerated the drug gradually by increasing the temperature using a Muffle furnace until free from carbonaceous materials, and obtained ash.

⇩

Cooled the crucible to room temperature.

⇩

Weighed the crucible containing the ash.

⇩

Calculated the percentage of total ash with reference to the initial drug sample weight.

$$\%\ Total\ ash = \frac{Ash\ weight}{Weight\ of\ sample} \times 100$$

2. Determination of acid insoluble ash[17]

The total ash obtained as above was boiled with 25ml of dilute HCl acid for 5 minutes.

⇩

The mixture was then filtered through the ashless filter paper.

⇩

The residue was washed with hot water and then ignited the tarred crucible using a Muffle Furnace. It was cooled and kept in a desiccator.

⇩

The ash obtained after incineration was weighed, and the acid insoluble ash of the drug was calculated with reference to the sample.

$$\%\ Acid\ insoluble\ ash = \frac{Acid\ insoluble\ ash\ weight}{Weight\ of\ sample} \times 100$$

3. Determination of water soluble ash[18]

The total ash obtained as above was boiled with 25ml of water for 5 minutes.

⇩

Then, it was filtered through the ashless filter paper.

⇩

The residue was washed with hot water, and then the tarred crucible was ignited using a Muffle Furnace. It was cooled and kept in desiccators.

⇩

The ash obtained after incineration was weighed, and the water-soluble ash of the drug was calculated with reference to the air-dried sample.

$$\% \text{ Wate soluble ash } = \frac{\text{Total ash weight} - \text{water insoluble residue in total ash}}{\text{Weight of sample}} \times 100$$

4. Loss on drying (LOD)[19]

2 grams of accurately weighed coarsely powdered drug were placed in a previously weighed porcelain dish.

⇩

It was dried in an oven at a temperature of 100-105°C.

⇩

Drying continued until two consecutive weights did not differ by more than 5mg.

⇩

Then, it was cooled in a desiccator.

⇩

The loss in weight of the material was calculated in %w/w of the sample.

➢ Here is the formula for calculation LOD as per USP:

$$LOD = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

Where:

W1=Weight of empty porcelain dish

W2=Weight of sample + porcelain dish

W3=Weight after drying

5. Swelling Index[20]

1 gram of the dried and powdered Marshmallow was taken.

⇩

It was transferred into a graduated cylinder.

⇩

Then, 25 mL of distilled water was added.

⇩

The mixture was left to set for about 24 hours at room temperature.

↓

The end-volume of the swollen material was determined directly in a graduated cylinder.

$$\text{Swelling Index } = \frac{(\text{Volume of swollen material (mL)} - \text{Original volume of liquid (mL)})}{\text{Weight of dry material (g)}}$$

Formulation of Ointment

Preparation of Extract from Black Seed (Nigella Sativa)[21]

The seeds were dried to remove the moisture content. The seeds were powdered and extraction was done with petroleum ether to remove the lipid contents and then was extracted with methanol by cold maceration process. The extract was concentrated by evaporating the extraction solvent.

Preparation of Extract from Marshmallow (Althaea Officinalis)[22]

20 grams of Althaea Officinalis (Marshmallow) powder were dissolved in 500 mL of boiled water. This mixture was then heated for 30 minutes at 70–75°C and filtered twice. The resulting liquid mucilage was subsequently heated using a water bath at 75°C for 24–42 hours. After the solvent evaporated, the dried mucilage was carved with a scalpel. The dried extract was then stored for further use in the preparation of the ointment.

Preparation of Ointment Base[23]

Initially ointment base was prepared by weighing accurately hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment.

Preparation of Herbal Ointment[24]

Herbal Ointment was prepared by mixing accurately weighed Nigella sativa and Althaea officinalis extract to the Ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more bases until to form homogenous ointment, finally transferred in a suitable container.

Table 4: Formulation of all batches.

Ingredients	**Quantity (% w/w)**				
	F1	**F2**	**F3**	**F4**	**F5**
Nigella sativa extract	5	5	5	5	5
Althaea officinalis extract	5	5	5	5	5
White soft paraffin	85	80	75	70	65
Cetostearyl alcohol	5	5	5	5	5
Hard paraffin	12	10	8	6	4
Wool fat	5	5	5	5	5

Evaluation of Ointment

1. Physical Properties of Herbal Ointment

The formulated ointment is evaluated for its organoleptic properties such as colour, odour, texture, homogeneity, phase separation and state of formulation.

2. Determination of pH[25]

The pH of the prepared ointment was measured utilizing calibrated digital pH meter by taking about 2.5 g of formulation in dry beaker and adding 50 ml of water to it. Beaker was then heated on water bath at temperature of 60–70°C. Then pH is measured for all six formulations as per specified procedures.

3. Determination of Spreadability[26]

Spreadability was assessed by placing an excess of the sample between two slides, which were then compressed to achieve a consistent thickness. The duration taken for the two slides to be separated was recorded. Spreadability was calculated by following formula:

$$S = \frac{M \times L}{T}$$

Where; S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

4. Extrudability[27]

The extrudability test evaluates the force necessary to extrude the ointment from a collapsible tube under specific pressure. It determines the percentage of ointment extruded from the tube upon the application of a defined force.

The extrudability of prepared Nigella sativa and Althaea officinalis containing ointment was calculated by using following formula:

$$Extrudability = \frac{Amount\ of\ ointment\ extruded\ from\ the\ collapsible\ tube}{Total\ amount\ of\ ointment\ filled\ in\ the\ collapsible\ tube} \times 100$$

5. Solubility[28]

Solubility in boiling water, alcohol, ether and chloroform was checked.

6. Viscosity[29,30]

The viscosity of the prepared ointment was assessed using a Brookfield viscometer. The measurements were taken using spindle number 64 at speeds of 50 and 100 RPM.

Relevant literature suggests a reciprocal correlation between the viscosity of ointments and the quantity of the released drugs.

7. Stability[31]

The stability of the formulated ointment was tested over a period of four weeks at various temperatures: 5±3°C, 25±2°C, and 37±2°C.

RESULTS AND DISCUSSION

1. Physicochemical analysis of *Althea Officials* and *Nigella Sativa*

Physicochemical analyses of *Althea Officinalis* and *Nigella Sativa* were conducted as part of preformulation studies, and the obtained values were recorded in the corresponding column of Table 5.

Table 5: Physicochemical analysis of *Althea Officials* and *Nigella Sativa.*

Sr. No.	Test	*Althaea officinalis* $Mean \pm SD$ (%)	*Nigella Sativa* $Mean \pm SD$ (%)
1	Total ash	4.2±0.32	4.88±0.26
2	Acid insoluble ash	1.6±0.18	0.46±0.03
3	Water soluble ash	9.3±0.56	3.07±0.16
4	Loss on drying (LOD)	5.8±0.38	3.55±0.23%
5	Swelling Index	6.4±0.42	NA

Total ash is useful for detecting low-quality products, exhausted drugs, and sandy or earthy matter present within the drug. Acid-insoluble ash detects the presence of silica and oxalates in the drugs. Water-soluble ash represents the portion of ash that dissolves in water, leaving behind insoluble residue when the herbal material is incinerated. The loss on drying parameter indicates the moisture content and volatile matter present in the herbal material.[32]

The Swelling Index is the ability to absorb water or other fluids, growing in volume but not dissolving. This property is critical in the study of polymers, hydrogels, and other materials applied in drug delivery systems, wound dressings, and food products. A high swelling index means that the plant material has a relatively high potential to absorb water and hence swell.

This is generally observed in materials that have high mucilage content or hydrophilic polysaccharides. A low swelling index means that the sample has a poor ability to absorb and swell, implying that its content of mucilaginous or hydrophilic compounds is relatively small.[33]

2. Physical Properties of Ointment

The physical properties of the prepared ointment were assessed for all batches and corresponding observation for each batch is presented in table 6.

Table 6: Physical Properties of Ointment.

Formulation	State	Colour	Texture	Homogeneity	Phase separation	Odour
F1	Semi solid	Grayish Black	Smooth	Homogeneous	No	Characteristic
F2	Semi solid	Grayish Black	Smooth	Homogeneous	No	Characteristic
F3	Semi solid	Grayish Black	Smooth	Homogeneous	No	Characteristic
F4	Semi solid	Grayish Black	Smooth	Homogeneous	No	Characteristic
F5	Semi solid	Grayish Black	Smooth	Homogeneous	No	Characteristic

As shown in the above table 6, all batches of the ointment were exhibited a semi-solid state with a characteristic grayish-black color, a smooth texture, and homogeneity across all formulations. The absence of phase separation and the presence of a characteristic odor further indicate the successful integration of *Nigella sativa* and *Althaea officinalis* extracts into the ointment.

Figure 3: Formulated Ointments.

3. Determination of pH

The pH values of the ointment were measured for all batches under the specified conditions and the obtained values were provided in the corresponding column of the table 7.

Table 7: Determination of pH.

S.No.	Batch	pH (Mean±SD)
1	F1	6.16 ± 0.12
2	F2	6.49 ± 0.20
3	F3	6.65 ± 0.10
4	F4	6.71 ± 0.17
5	F5	6.83 ± 0.14

The pH range observed in these formulations (F1-F5) falls within the acceptable range for topical products. The pH of ointments plays a significant role in their stability, compatibility with the skin, and potential interaction with other components. The slight variations in pH among formulations could be attributed to minor differences in the concentration of ingredients. However, these variations are within an acceptable range and are unlikely to impact the overall performance or safety of the ointment.

Figure 4: pH Measurement.

4. Spreadability

The spreadability for all batches was determined, and the obtained values were recorded in the table respective column of the table 8.

Table 8: Determination of Spreadability.

Sr. No.	Batch	Spreadability (gm.cm/sec) (Mean±SD)
1	F1	7.74 ± 0.80
2	F2	13.23 ± 0.51
3	F3	17.17 ± 0.60
4	F4	24.61 ± 1.15
5	F5	29.12 ± 1.30

Spreadability is a crucial factor for patient compliance and ensures uniform distribution of the ointment on a large skin area. F5 demonstrates the highest spreadability value of 29.12 ± 1.30 gm.cm/sec, suggesting that this formulation exhibits the easiest spreadability and could potentially provide better coverage over the skin surface.

5. Extrudability

The extrudability of the ointment was evaluated, and the results showed in the table 9.

Table 9: Extrudability.

S.No.	Batch	Extrudability (%) (Mean±SD)
1	F1	80.2 ± 1.27
2	F2	85.1 ± 0.86
3	F3	87.8 ± 1.26
4	F4	90.2 ± 1.53
5	F5	95.8 ± 2.10

Extrudability is the ability of a substance to be extruded out of its container, typically measured as percentage of the total amount of substance within the container. Extrudability is a crucial parameter as it determines the ease with which the ointment can be dispensed and applied by the user. F5 exhibited highest extrudability compared to others. This suggests that the concentration of ingredients used for this formulation may be more favorable in terms of extrudability.

6. Solubility

The solubility of the formulated ointment was evaluated in various solvents. All the batches were partially soluble in boiling water and highly soluble in alcohol.

Partial solubility could indicate the presence of both hydrophobic and hydrophilic components within the formulation. It also indicates that water alone is not the most effective solvent for the complete dispersion of the ointment. High solubility of the ointment in alcohol is a noteworthy result. Alcohol is common solvent in pharmaceutical formulations and offers advantages in terms of extraction of active ingredients.

7. Viscosity

The viscosity of the ointment was measured for each batch, and the obtained values were recorded in the table 10.

Table 10: Viscosity.

Sr. No.	Batch	RPM	Viscosity (cP) (Mean±SD)
1	F1	50	
		100	
2	F2	50	
		100	
3	F3	50	
		100	
4	F4	50	
		100	
5	F5	50	
		100	

Viscosity measurements values indicated that as the concentration of hard paraffin decreases from F1 to F5, there is a corresponding decrease in viscosity. Measurement was carried out using spindle number 64 at speeds of 50 RPM and 100 RPM to observe the effect of changing RPM on Viscosity. Viscosity may affect spreadability, ease of application and ointment's ability to adhere to the skin. Hence, it potentially impact consumer experience and product efficacy.

Figure 5: Brookfield Viscometer.

8. Stability Study

A stability study was performed on all six batches under specified conditions and the results of study are given in the table 11.

Table 11: Stability study.

S.No.	Batch	Stability study		
1	F1	Unstable	Stable	Stable
2	F2	Stable	Stable	Stable
3	F3	Stable	Stable	Stable
4	F4	Stable	Stable	Stable
5	F5	Stable	Stable	Stable

Stability studies indicated that F2, F3, F4 and F5 were remained stable under different temperature conditions (i.e. 5±3 °C, 25±2 ℃, 37±2 ℃) over a four-week period.

The term "stable" indicates that the ointment maintained its physical, chemical, and microbiological integrity throughout the duration of the study under the given temperature conditions. This stability is a crucial aspect of pharmaceutical products, ensuring that they remain effective and safe for use over their intended shelf life.

CONCLUSION

The project explored on the herbal-based wound therapy by using combined potential of Nigella sativa and Althaea officinalis extracts. The formulated ointment exhibited favorable physical properties, pH levels, spreadability, extrudability, viscosity, and stability. Based on the observed results, F5 appears to be the best ointment formulation. The present experimental study demonstrated the successful development and evaluation of combined formulation of Nigella sativa and Althaea officinalis extract ointment. The results of this study pave the way for advancements in herbal-based therapies for wound healing. Further research and clinical trials are needed to fully understand their potential and optimize their use in clinical practice.

REFERENCES

1. Grey JE, Enoch S, Harding KG. Wound assessment. BMJ., 2006; 332(7536): 285-288.
2. Cialdai F, Risaliti C, Monici M. Role of fibroblasts in wound healing and tissue remodeling on Earth and in space. Front Bioeng Biotechnol, 2022; 10: 958381.
3. Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol, 2016; 5: 614-620.

4. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med., 2014; 6(265).
5. Mohd Zaid MH, Hanafi MF, Haris MS. A review of black seed extract as an agent in the wound healing process. J Pharm., 2021; 1(2): 87-96.
6. Rezaei M, Dadgar Z, Noori-Zadeh A, Mesbah-Namin SA, Pakzad I, Davodian E. Evaluation of the antibacterial activity of the Althaea officinalis L. leaf extract and its wound healing potency in the rat model of excision wound creation. Avicenna J Phytomed, 2015; 5(2): 105-112.
7. Palanisamy CP, Alugoju P, Jayaraman S, Poompradub S. Nigella sativa L. seed extracts promote wound healing progress by activating VEGF and PDGF signaling pathways: An in vitro and in silico study, 2023; 12: 436.
8. Nourbar E, Mirazi N, Yari S, Rafieian‑Kopaei M, Nasri H. Effect of hydroethanolic extract of Nigella sativa L. on skin wound healing process in diabetic male rats. Int J Prev Med., 2019; 10: 18.
9. Sallehuddin N, Nordin A, Idrus RBH, Fauzi MB. Nigella sativa and Its Active Compound, Thymoquinone, Accelerate Wound Healing in an In Vivo Animal Model: A Comprehensive Review. Int J Environ Res Public Health, 2020; 17(11): 4160.
10. Rezaei M, Dadgar Z, Noori-Zadeh A, Mesbah-Namin SA, Pakzad I, Davodian E. Evaluation of the antibacterial activity of the Althaea officinalis L. leaf extract and its wound healing potency in the rat model of excision wound creation. Avicenna J Phytomed, 2015; 5(2): 105-112.
11. Bonaterra GA, Bronischewski K, Hunold P, Schwarzbach H, Heinrich EU, Fink C, et al. Anti-inflammatory and Anti-oxidative Effects of Phytohustil and Root Extract of Althaea officinalis L. on Macrophages in vitro. Front Pharmacol, 2020; 11: 290.
12. Dev Kaushal et al. International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Oct. 2022; 7(10): 30-38.
13. Shelke UY, Mahajan AA. Review on: an Ointment. IJPPR, 2015; 4(2): 178-187.
14. Aulton ME. Pharmaceutics: The Science of Dosage Form Design. 2nd ed. Churchill Livingstone, 1988; 529.
15. Kaushal D, Upadhyaya N. International Journal of Pharmaceutical Sciences & Medicine (IJPSM), 2022; 7(10): 30-38.

16. Thilakarathna RCN, Madhusankha GDMP, Navaratne SB. Comparison of physico-chemical properties of Indian and Ethiopian origin Black cumin (Nigella sativa) seed cake. Int J Food Sci Nutr., 2018; 3(4): 30-31.
17. Ahmad A, Husain A, Mujeeb M, et al. Physicochemical and phytochemical standardization with HPTLC fingerprinting of Nigella sativa L. seeds. Pak J Pharm Sci., 2014; 27(5): 1175-1182.
18. Husain M, Wadud A, Hamiduddin, et al. Physicochemical standardization of mucilage obtained from Althaea officinalis Linn – Root. Phcog Mag., 2019; 15: S155-S161.
19. Bhalani U, Shah K. Preparation and evaluation of topical gel of Nigella sativa (Kalonji). Int J Res Dev Pharm L Sci., Jun-Jul, 2015; 4(4): 1669-1672.
20. Rezaei M, Dadgar Z, Noori-Zadeh A, et al. Evaluation of the antibacterial activity of the Althaea officinalis L. leaf extract and its wound healing potency in the rat model of excision wound creation. Avicenna J Phytomed, 2015; 5(2): 105-112.
21. Chaudhari RG, Barhewar PA, Parshuramkar YT, Bhange P. Formulation and evaluation of herbal ointment containing Salacia oblonga and Curcuma long extracts. IJPBSTM, 2020; 10(1): 111-115.
22. Chhetri HP, Shrestha Y, Yogol N, Sherchan J, K.C. A, Mansoor S, et al. Formulation and evaluation of antimicrobial herbal ointment. KU J Sci Eng Technol, 2010; 6(1): 102-107.
23. Maru AD, Lahoti SR. Formulation and evaluation of ointment containing sunflower wax. Asian J Pharm Clin Res., 2019; 12(8): 116-120.
24. Prasad LS, Gurunath KP, Chandrasekar SB, Umashankar C, Pawar AT. Formulation and evaluation of herbal formulations (Ointment, Cream, Gel) containing Tridax procumbens and Areca catachu. J Sci Innov Res., 2017; 6(3): 97-100.
25. Sakthivel M, Halith SM, Karthikeyan R, Kaviya M, Kiruthika M, Kowsalya S, et al. Formulation and evaluation of herbal ointment containing Neem and Turmeric Extract. Int J Pharm Sci Rev Res., 2023; 78(2): 134-139.
26. Shaikh SC, Sanap D, Bhusari DV, Jain S, Kochar PP, Memon FS. Fabrication and evaluation of herbal ointment formulations of Moringa oleifera for topical delivery. Univ J Pharm Res., 2018; 3(4): 37-41.
27. Sawant SE, Tajane MD. Formulation and evaluation of herbal ointment containing Neem and Turmeric extract. J Sci Innov Res., 2016; 5(4): 149-151.
28. Prasad LS, Gurunath KP, Chandrasekar SB, Umashankar C, Pawar AT. Formulation and evaluation of herbal formulations (Ointment, Cream, Gel) containing Tridax procumbens and Areca catachu. J Sci Innov Res., 2017; 6(3): 97-100.

29. Pawar RS. Development, optimization and evaluation of different herbal formulations for wound healing. J Wound Care., 2015; 7(3): 447-452.
30. Erös I, Soósné-Csányi E, Selmeczi B. A viszkozitás hatása kenöcsök, krémek, gélek és emulziók gyógyszerleadására [Influence of viscosity on drug release from ointments, creams, gels and emulsions]. Acta Pharm Hung, 1994; 64(2): 57-61.
31. Chaudhari RG, et al. Formulation and Evaluation of Herbal Ointment Containing Salacia oblonga and Curcuma longa Extracts. IJPBSTM, 2020; 10(1): 111-115.
32. Al-Snafi AE. The pharmaceutical importance of Althaea officinalis and Althaea rosea: a review. Int J PharmTech Res., 2013; 5(3): 1378-1385.
33. Ruan B, Jacobi AM. Ultrasonication effects on thermal and rheological properties of carbon nanotube suspensions. Nanoscale Res Lett., 2012; 7: 27-41.